03-18-02

JC05 Rec'd PCT/PTO 15 MAR 2002

JC962 U.S. PTO 03/15/02

FORM PTO-1390 DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE (REV 1-98)		ATTORNEY'S DOCKET NO. DEBE:007US
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 10/088549
INTERNATIONAL APPLICATION NO. PCT/EP 00/09130	INTERNATIONAL FILING DATE September 18, 2000	PRIORITY DATE CLAIMED September 16, 1999

TITLE OF INVENTION
ASSAY TO DETECT SUBSTANCES USEFUL FOR THERAPY

APPLICANT(S) FOR DO/EO/US
Erik Nielsen, Savvas Christophoridis, Carol Murphy, Marino Zerial and Stefano De Renzis

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is transmitted herewith (required only if not transmitted by the International bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 16 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information: PCT Publication A1 OF WO 01/20022 which comprises the application papers as filed and the ISR; PCT request; PCT Chapter II Demand; first written opinion of August 22, 2001; IPER of December 18, 2001; Statement as Required Under 37 C.F.R. § 1.821(f); Diskette and Postcard.

EXPRESS MAIL MAILING LABEL
EXPRESS MAIL NO.: EL794535160US
DATE OF DEPOSIT: MARCH 15, 2002

JC10 Rec'd PCT/PTO 15 MAR 2002

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 10/088549	INTERNATIONAL APPLICATION NO. PCT/EP 00/09130	ATTORNEY'S DOCKET NUMBER DEBE:007US

17. ☒ The following fees are submitted:

Basic National Fee (37 CFR 1.492(a)(1)-(5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....$1,000.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....$ 860.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....$ 710.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4).....$ 690.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(2)-(4).....$ 100.00

CALCULATIONS PTO USE ONLY

ENTER APPROPRIATE BASIC FEE AMOUNT =				$1,000.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$ .00	
Claims	Number Filed	Number Extra	Rate		
Total Claims	26 - 20 =	6	x $ 18.00	$108.00	
Independent Claims	1 - 2 =	0	x $ 80.00	$ .00	
Multiple dependent claim(s) (if applicable)			+ $270.00	$ .00	
TOTAL OF ABOVE CALCULATIONS =				$1,108.00	
Reduction by ½ for filing by small entity, if applicable. Applicant is entitled to small entity status pursuant to 37 CFR 1.27.				$-0-.00	
SUBTOTAL =				$1,108.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$-0-.00	
TOTAL NATIONAL FEE =				$-0-.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property) +				$-0-.00	
TOTAL FEES ENCLOSED =				$1,108.00	
				Amount to be refunded:	$ .00
				charged:	$ .00

a. ☐ A check in the amount of $_____ cover the above fees is enclosed.

b. ☒ Please charge Deposit Account No. 50-1212/10201173/SLH in the amount of $ 1,108.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 50-1212/10201173/SLH. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

STEVEN L. HIGHLANDER, ESQ.
FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
512.474.5201

SIGNATURE

Steven L. Highlander
NAME

37,642
REGISTRATION NUMBER

EXPRESS MAIL MAILING LABEL
EXPRESS MAILING NO.: EL794535160US
DATE OF DEPOSIT: MARCH 15, 2002

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Erik Neilsen, Savvas Christophoridis, Carol Murphy, Marino Zerial and Stefano De Renzis

Serial No.: Unknown

Filed: March 14, 2002

For: ASSAY TO DETECT SUBSTANCES USEFUL FOR THERAPY

Group Art Unit: Unknown

Examiner: Unknown

Atty. Dkt. No.: DEBE:007US/SLH

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Please consider the following amendments prior to examination of the above-captioned application. It is believed that no fees are occasioned by this filing; however, should any fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason, the Commissioner is authorized to deduct said fees from Fulbright & Jaworski L.L.P. Account No.: 50-1212/10201173/DEBE:007US/SLH. Please date stamp and return the enclosed postcard as evidence of receipt.

AMENDMENT

In the Specification

Please insert the following paragraph after line 1 of page 1:

This application claims priority to PCT/EP 00/09130, filed on September 18, 2000, and EP 99 118 385.6, filed March 22, 2001. The entire content of both these applications are incorporated by reference.

In the Claims

Please cancel claims 1-13, without prejudice or disclaimer.

Please add the following claims:

14. (New) A method of screening a substance for use as pharmaceutical agents for the prophylaxis and/or treatment of a proliferative, invasive or cell migration disorder comprising assessing the affect of said substance on a GTPase-GTPase effector interaction.

15. (New) The method of claim 14, wherein the GTPase is of the Rab family.

16. (New) The method of claim 14, wherein the GTPase is Rab4, Rab5, Rab7, Rab11, Rab17, Rab18, or Rab22.

17. (New) The method of claim 14, wherein the disorder is selected from the group consisting of cancer, endometriosis, atherosclerosis, inflammatory disease, allergic disease, infectious diseases, diabetes, Alzheimer's disease, and skin repair disease.

18. (New) The method of claim 17, wherein the infectious disease is AIDS, tuberculosis, pseudotuberculosis, cholera, malaria, gastroenteritis, enteric fever, or typhus.

19. (New) The method of claim 17, wherein the infectious disease is caused by Mycobacterium, Staphylococcus, Toxoplasma, Trypanosoma, Listeria, Salmonella, Legionella, Leishmania, Coxiella, Shigella, Yersinia, Neisseria, Vibrio, or Bartonella

20. (New) The method of claim 17, wherein the infectious disease is caused by an infectious agent that infects cells by the endocytic route and resides intracellularly in phagosomes escaping the cellular killing mechanisms.

21. (New) The method of claim 17, wherein the cancer is a benign tumor, a malignant tumor, a carcinoma, a sarcoma, a melanoma, a leukemia, a glioma, or a neuroblastoma, in particular a lung carcinoma, an osteosarcoma, a lymphoma, a soft tissue sarcoma, a breast carcinoma, a bile cancer, a cervix carcinoma, a cancer of the (small) intestine, of the kidneys, of the cavity of the mouth, a penis carcinoma, an ovary cancer, a stomach cancer, a cancer of the tongue, a brain cancer, a bladder carcinoma, a prostate carcinoma, a liver carcinoma, a carcinoma of the pancreas, and every tumor that invades other tissues and organs distinct from its site of origin.

22. (New) The method of claim 14, wherein the assay is carried out in the presence of a labeled GTPase effector/regulator molecule.

23. (New) The method of claim 22, wherein the label is a fluorescent or radiactive label.

24. (New) The method of claim 14, wherein assessing comprises determining GTPase function.

25. (New) The method of claim 14, wherein assessing comprises determining GTPase interaction with a GTPase effector/regulator molecule.

26. (New) The method of claim 24, wherein GTPase function is determined by measuring GTP/GDP nucleotide exchange, GTP hydrolysis, endosomal motility, and endosomal trafficking.

27. (New) The method of claim 25, wherein a GTPase effector molecule is bound to a substrate.

28. (New) The method of claim 27, wherein the substrate is a chromatographic matrix or a bead.

29. (New) The method of claim 14, wherein the substance comprises one or more of the following functional groups: a halide atom bound to an alkyl, alkenyl, alkinyl or aryl residue, an alcohol group (primary, secondary, tertiary), an ether group, a carbonyl function (aldehyde or ketone), a carboxylic acid group, a carboxylic anhydride group, a carbamoyl group, a haloformyl group, a cyano group, an ester group including a lactone group, a benzyl, phenyl, tolyl, tosyl, sulfonyl group, an amino group (primary, secondary, tertiary), a sterol moiety, an isocyanate, a cyanate, a thioisocyanate, a thiocyanate, a carbamate, an azide, a diazo group, or a quinone group.

30. (New) The method of claim 14, wherein the substance is an organometallic compound, a β-hydroxy carboxylic acid, an inorganic acid or complex such as a metallocene, a nucleic acid.

31. (New) The method of claim 30, wherein the antibody is a polyclonal or monoclonal antibody, or a fragment thereof, a humanised or human antibody, an inhibitory or stimulatory antibody.

32. (New) The method of claim 14, wherein the substance is a protein or peptide.

33. (New) The method of claim 32, wherein the protein is a cytokine, a hormone, or an antibody.

34. (New) The method of claim 32, wherein the peptide is an oligopeptide comprising up to 20 amino acid residues

35. (New) The method of claim 34, wherein the oligopeptide is about 8, about 10 or about 12 amino acid residues in length.

36. (New) The method of claim 14, wherein the substance is a nucleic acid.

37. (New) The method of claim 36, wherein the nucleic acid is genomic DNA, cDNA, or mRNA, an oligonucleotide, or an oligoribonucleotide, wherein said nucleic acid encodes all or a fragment of a proteinaceous GTPase effector.

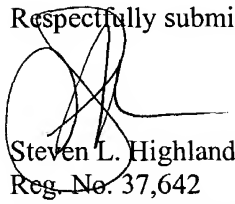
38. (New) The method of claim 37, wherein the encoding sequence is SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, or and 15.

39. (New) The method of claim 37, wherein the nucleic acid further comprises a gene therapy vector.

REMARKS

Should the examiner have any questions regarding the content of this preliminary amendment, a telephone call to the undersigned is invited.

Respectfully submitted,



Steven L. Highlander
Reg. No. 37,642
Attorney for Erik Nielson, Savvas Chritophoridis, Carol Murphy, Marino Zerial and Stefano De Renzis

FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
(512) 536-3184

Date: March 15, 2002

MARKED UP COPY OF CLAIMS

1. (Canceled) Use of an effector of a GTPase as a target in an *in vitro* or *in vivo* assay to detect substances useful as pharmaceutical agents for the prophylaxis and/or treatment of cancer and other proliferative, invasive or cell migration disorders such as endometriosis, atherosclerosis, inflammatory and allergic diseases, infectious diseases, diabetes, Alzheimer's disease and skin repair diseases such as psoriasis.

2. (Canceled) Use of claim 1, wherein the GTPase is of the Rab family.

3. (Canceled) Use of claim 2, wherein the GTPase is any of Rab4, Rab5, Rab7, Rab11, Rab17, Rab18, and Rab22.

4. (Canceled) Use of any of the preceding claims, wherein the infectious disease is AIDS, tuberculosis, pseudotuberculosis, cholera, malaria, gastroenteritis, enteric fever, typhus, those diseases caused by pathogens (bacteria or organisms) such as Mycobacterium, Staphylococcus, Toxoplasma, Trypanosoma, Listeria, Salmonella, Legionella, Leishmania, Coxiella, Shigella, Yersinia, Neisseria, Vibrio, Bartonella, or any other infectious disease caused by an infectious agent that infects cells by the endocytic route and resides intracellularly in phagosomes escaping the cellular killing mechanisms.

5. (Canceled) Use of any of claims 1 to 3, wherein the cancer is a benign tumor, a malignant tumor, a carcinoma, a sarcoma, a melanoma, a leukemia, a glioma, or a neuroblastoma, in particular a lung carcinoma, an osteosarcoma, a lymphoma, a soft tissue sarcoma, a breast carcinoma, a bile cancer, a cervix carcinoma, a cancer of the (small) intestine, of the kidneys, of the cavity of the mouth, a penis carcinoma, an ovary cancer, a stomach cancer, a cancer of the tongue, a brain cancer, a bladder carcinoma, a prostate carcinoma, a liver carcinoma, a carcinoma of the pancreas, and every tumor that invades other tissues and organs distinct from its site of origin.

6. (Canceled) Use of any of the preceding claims, wherein the assay is carried out in the presence of one or more GTPase effector/regulator molecule(s) which is/are either native

and biochemically purified from a vertebrate, or recombinant and biochemically purified from bacterial cultures, from yeast cultures, or from other cultured eukaryotic cells, in either case labeled by a covalent modification or radioactivity suitable for use in the assay.

7. (Canceled) Use of claim 6, wherein the assay is carried out in the simultaneous presence of at least one type of GTPase and/or endosomal membrane fractions fluorescently labeled or labeled by any other modification that allows its detection, and/or cytosolic extracts, and/or an ATP-regenerating system and/or a number of chemicals to be tested for their suitability as an anti-cancer or anti-infectious diseases drug.

8. (Canceled) Use of any of the preceding claims, wherein the substance useful as pharmaceutical agent is a molecule/substance that carries one or more of the following functional groups: a halide atom bound to an alkyl, alkenyl, alkinyl or aryl residue, an alcohol group (primary, secondary, tertiary), an ether group, a carbonyl function (aldehyde or ketone), a carboxylic acid group, a carboxylic anhydride group, a carbamoyl group, a haloformyl group, a cyano group, an ester group including a lactone group, a benzyl, phenyl, tolyl, tosyl, sulfonyl group, an amino group (primary, secondary, tertiary), an isocyanate, a cyanate, a thioisocyanate, a thiocyanate, a carbamate, an azide, a diazo group, and a quinone group; or is an organometallic compound, a sterol moiety(ies)-containing molecule, a β-hydroxy carboxylic acid, an inorganic acid or complex such as a metallocene, a nucleic acid, a cytokine, a hormone, an antibody, or an oligopeptide comprising up to 20, preferably 8, 10, or 12, amino acid residues.

9. (Canceled) Use of claim 8, wherein the antibody is a polyclonal or monoclonal antibody, or a fragment thereof, humanised or human, inhibitory or stimulatory, raised against and targeted towards any of the aforementioned GTPase effectors.

10. (Canceled) Use of claim 8, wherein the nucleic acid is genomic DNA, cDNA, or mRNA, or a fragment there, an oligonucleotide, an oligoribonucleotide, all being based on or derived from any of the GTPase effector having any of the sequences as depicted in SEQ

ID NO: 1, 3, 5, 7, 9, 11, 13, and 15, or gene therapy vectors derived from the aforementioned GTPase effector gene sequences.

11. (Canceled) A kit useful for carrying out the assay of any of the preceding claims, the kit comprising in a suitable means at least one GTPase effector/regulator molecule which is either native and biochemically purified from a vertebrate, or recombinant and biochemically purified from bacterial cultures, from yeast cultures, or from other cultured eukaryotic cells, in either case labeled by a covalent modification or by radioactivity suitable for the use in the assay.

12. (Canceled) The kit of claim 11, wherein the kit further comprises at least one type of GTPase and/or endosomal membrane fractions fluorescently labeled or labeled by any other modification that allows its detection, and/or cytosolic extracts, and/or an ATP-regenerating system and/or a number of chemicals to be tested for their suitability as a drug effective against any of the diseases depicted in claims 1, 4, and 5.

13. (Canceled) The kit of claim 12, wherein the at least one GTPase is/are one or more of the GTPases Rab4, Rab5, Rab7, Rab11, Rab17, Rab18, and Rab22.

Please add the following claims:

14. (New) A method of screening a substance for use as pharmaceutical agents for the prophylaxis and/or treatment of a proliferative, invasive or cell migration disorder comprising assessing the affect of said substance on a GTPase-GTPase effector interaction.

15. (New) The method of claim 14, wherein the GTPase is of the Rab family.

16. (New) The method of claim 14, wherein the GTPase is Rab4, Rab5, Rab7, Rab11, Rab17, Rab18, or Rab22.

17. (New) The method of claim 14, wherein the disorder is selected from the group consisting of cancer, endometriosis, atherosclerosis, inflammatory disease, allergic disease, infectious diseases, diabetes, Alzheimer's disease, and skin repair disease.

18. (New) The method of claim 17, wherein the infectious disease is AIDS, tuberculosis, pseudotuberculosis, cholera, malaria, gastroenteritis, enteric fever, or typhus.

19. (New) The method of claim 17, wherein the infectious disease is caused by Mycobacterium, Staphylococcus, Toxoplasma, Trypanosoma, Listeria, Salmonella, Legionella, Leishmania, Coxiella, Shigella, Yersinia, Neisseria, Vibrio, or Bartonella

20. (New) The method of claim 17, wherein the infectious disease is caused by an infectious agent that infects cells by the endocytic route and resides intracellularly in phagosomes escaping the cellular killing mechanisms.

21. (New) The method of claim 17, wherein the cancer is a benign tumor, a malignant tumor, a carcinoma, a sarcoma, a melanoma, a leukemia, a glioma, or a neuroblastoma, in particular a lung carcinoma, an osteosarcoma, a lymphoma, a soft tissue sarcoma, a breast carcinoma, a bile cancer, a cervix carcinoma, a cancer of the (small) intestine, of the kidneys, of the cavity of the mouth, a penis carcinoma, an ovary cancer, a stomach cancer, a cancer of the tongue, a brain cancer, a bladder carcinoma, a prostate carcinoma, a liver carcinoma, a carcinoma of the pancreas, and every tumor that invades other tissues and organs distinct from its site of origin.

22. (New) The method of claim 14, wherein the assay is carried out in the presence of a labeled GTPase effector/regulator molecule.

23. (New) The method of claim 22, wherein the label is a fluorescent or radiactive label.

24. (New) The method of claim 14, wherein assessing comprises determining GTPase function.

25. (New) The method of claim 14, wherein assessing comprises determining GTPase interaction with a GTPase effector/regulator molecule.

26. (New) The method of claim 24, wherein GTPase function is determined by measuring GTP/GDP nucleotide exchange, GTP hydrolysis, endosomal motility, and endosomal trafficking.

27. (New) The method of claim 25, wherein a GTPase effector molecule is bound to a substrate.

28. (New) The method of claim 27, wherein the substrate is a chromatographic matrix or a bead.

29. (New) The method of claim 14, wherein the substance comprises one or more of the following functional groups: a halide atom bound to an alkyl, alkenyl, alkinyl or aryl residue, an alcohol group (primary, secondary, tertiary), an ether group, a carbonyl function (aldehyde or ketone), a carboxylic acid group, a carboxylic anhydride group, a carbamoyl group, a haloformyl group, a cyano group, an ester group including a lactone group, a benzyl, phenyl, tolyl, tosyl, sulfonyl group, an amino group (primary, secondary, tertiary), a sterol moiety, an isocyanate, a cyanate, a thioisocyanate, a thiocyanate, a carbamate, an azide, a diazo group, or a quinone group.

30. (New) The method of claim 14, wherein the substance is an organometallic compound, a β-hydroxy carboxylic acid, an inorganic acid or complex such as a metallocene, a nucleic acid.

31. (New) The method of claim 30, wherein the antibody is a polyclonal or monoclonal antibody, or a fragment thereof, a humanised or human antibody, an inhibitory or stimulatory antibody.

32. (New) The method of claim 14, wherein the substance is a protein or peptide.

33. (New) The method of claim 32, wherein the protein is a cytokine, a hormone, or an antibody.

34. (New) The method of claim 32, wherein the peptide is an oligopeptide comprising up to 20 amino acid residues

35. (New) The method of claim 34, wherein the oligopeptide is about 8, about 10 or about 12 amino acid residues in length.

36. (New) The method of claim 14, wherein the substance is a nucleic acid.

37. (New) The method of claim 36, wherein the nucleic acid is genomic DNA, cDNA, or mRNA, an oligonucleotide, or an oligoribonucleotide, wherein said nucleic acid encodes all or a fragment of a proteinaceous GTPase effector.

38. (New) The method of claim 37, wherein the encoding sequence is SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, or and 15.

39. (New) The method of claim 37, wherein the nucleic acid further comprises a gene therapy vector.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Erik Nielsen, et al.

Serial No.: 10/088,549

Filed: March 15, 2002

For: ASSAY TO DETECT SUBSTANCES USEFUL FOR THERAPY

Group Art Unit: Unknown

Examiner: Unknown

Atty. Dkt. No.: DEBE:007US

EXPRESS MAIL MAILING LABEL	
NUMBER	EL 503180194 US
DATE OF DEPOSIT	August 2, 2002

SECOND PRELIMINARY AMENDMENT

BOX PCT
Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants respectfully submit this Preliminary Amendment in the above-referenced case. Consideration of this case in view of the amendments made herein is respectfully requested.

AMENDMENT

The Preliminary Amendment filed March 15, 2002 by the Applicants (copy enclosed) contained an error. An Amendment to the previously filed Preliminary Amendment is hereby requested.

On page 1 of the Specification, please delete the paragraph spanning lines 1 through 3 previously added by the Preliminary Amendment of March 15, 2002 and replace it with the following paragraph:

--This application claims priority to PCT/EP00/09130 filed on September 18, 2000, and EP 99 118 385.6, filed September 16, 1999. The entire content of both these applications are incorporated by reference.--

REMARKS

The specification has been amended to delete the incorrect priority data previously added by a Preliminary Amendment and to recite the correct priority data.

Should any fees under 37 C.F.R. §§ 1.16 to 1.21 be required, the Commissioner is hereby authorized to deduct said fees from Fulbright & Jaworski Deposit Account No. 50-1212/DEBE:007US.

The Examiner is invited to contact the undersigned attorney with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

Reg No. 37,255 For

Steven L. Highlander
Reg. No. 37,642
Attorney for Applicants

FULBRIGHT & JAWORSKI L.L.P.
600 Congress Avenue, Suite 2400
Austin, Texas 78701
(512) 474-5201

Date: August 2, 2002

Rec'd PCT/PTO 15 MAR 2002
PCT/EP00/09130
10/088549

Assay to Detect Substances Useful for the Therapy

The present invention relates to the use of effectors/regulators for Rab GTPases in *in vitro* and *in vivo* assays that recapitulate and measure the role of these effectors/regulators in membrane transport and membrane-cytoskeleton interactions in the endocytic pathway as novel targets to find therapeutic drugs to prevent or inhibit cancer cell growth and arrest cancer cell invasiveness as well as for stimulating and/or restoring endocytic transport and phagosome maturation in cells infected by intracellular parasites, which drugs are therefore useful for the therapy and optionally also the prophylaxis of 1) cancer and other proliferative (skin repair diseases such as psoriasis), invasive or cell migration disorders (endometriosis, atherosclerosis, inflammation and allergic diseases), 2) infectious (bacterial and viral) diseases, 3) diabetes, 4) Alzheimer's disease. In addition, the present invention is also directed to kits useful as a means to detect drugs suitable as anti-cancer and anti-infectious diseases drugs.

A class of molecules shown to play an important role in the regulation of intracellular transport and organelle function is represented by Rab proteins, small GTPases of the Ras superfamily, which are required in virtually every transport step which has been investigated. Similar to other GTPases, these molecules use the conformational change induced by GTP hydrolysis to regulate downstream events necessary for vesicle formation, docking and fusion. In the GTP-bound, active form Rab proteins bind to effector proteins and in this way transmit their signal to the transport machinery. For example, in membrane docking and fusion, Rab proteins regulate the activity of SNAREs. SNAREs are integral membrane proteins that by pairing on opposite membranes engaged in docking lead to membrane fusion. The pairing of SNAREs requires the activity of Rab proteins and therefore of Rab effectors. For example, endosome membrane docking requires the presence of the Rab5 effector EEA1 which, upon bridging the two opposite membranes, allows SNAREs to pair in trans, thus leading to membrane fusion (Chistoforidis et al., 1999a). The role of Rab proteins is not restricted to membrane docking and fusion but recent data provide evidence that Rab proteins also regulate the association with and motility of vesicles along cytoskeletal filaments. Membrane-cytoskeleton interactions play an important role in

determining the intracellular distribution and motility of organelles and transport vesicles. The cytoskeleton is made of different types of filaments, actin filaments and microtubules being the best characterised. Actin filaments and microtubules undergo continuous remodelling to serve the needs of the cell in cell architecture, motility, and organelle movement. Microtubules play a pivotal role in cell division but also constitute tracks along which cargo is transported across long distances such as between the periphery and the centre of the cell, from the basal to the apical pole in epithelial cells, or along the axon in neurons. Actin filaments are well known to function in muscle contraction. They are also important for cell motility, cell shape and vesicular transport. Different proteins are necessary to attach the organelles and transport vesicles to, and move them along, these filaments. Rab5 has recently been demonstrated to regulate the attachment of endosomes to, and motility along, microtubules. This suggests that Rab5, and in general members of the Rab proteins family, regulate various aspects of intracellular transport, including the ability of organelles to move along cytoskeletal tracks.

The identification of a large number of effectors and regulators for the small GTPase Rab5 provides a molecular explanation for the multiplicity of functions of Rab5 and allow to predict similar mechanisms for other Rab GTPases. Rab5 regulates a molecular network of several effector proteins, each contributing a specific function in membrane organisation, vesicle formation, vesicle and organelle movement, membrane docking and fusion. Most importantly, by functioning in a cooperative fashion Rab5 effectors modify the membrane environment and thus contribute to the biogenesis of the early endosome membrane. This mode of action is exemplified by the following mechanism elucidated by the present inventors. Upon activation by the effector/exchange factor Rabaptin-5/Rabex-5 complex, Rab5 locally recruits and activates phosphoinositide PI3-Kinases, leading to the generation of PI(3)P and consequently allowing the membrane recruitment of other Rab5 effectors (e.g. EEA1; see below) that bind to both Rab5:GTP and PI(3)P (Christoforidis et al., 1999b). Furthermore, Rab5 effectors are engaged in the formation of oligomeric complexes on the early endosome membrane (McBride et al., 1999). By the same criteria, other Rab proteins present in the early endosomes would be expected to recruit multiple effectors within a separate membrane environment. Consistent with this, studies using (green fluorescent protein) GFP-tagged Rab5, Rab4, and Rab11 have demonstrated that these GTPases are present in separate sub-compartments of the early endosome membrane. Endosomes are therefore organised as a mosaic of different Rab-domains created through the recruitment of specific effector proteins, which co-operatively act to generate a topologically restricted and functionally specialized environment on the endosome membrane.

Laying at the core of the organelle biogenesis and membrane transport mechanisms, Rab GTPases and their effectors are placed in a strategic position to regulate and mediate the trafficking of cellular membrane and soluble components along the biosynthetic and endocytic pathways. Their function includes the regulation of transport of cellular constituents (proteins and lipids) that are implicated in pathological alterations. Consequently, the present inventors' understanding of the mechanisms underlying membrane trafficking and membrane-cytoskeleton interactions, has applications in biotechnological research. Applications are in particular possible for those diseases where modulation of the membrane trafficking properties of endosomes provides an opportunity for therapeutic intervention. The main research areas for which this knowledge has proven to have important implications are: 1) cancer and other proliferative (skin repair diseases such as psoriasis), invasive or cell migration disorders (endometriosis, atherosclerosis, inflammation and allergic diseases), 2) infectious (bacterial and viral) diseases, 3) diabetes, 4) Alzheimer's disease. In the following section, the Rab GTPases and Rab effectors machinery will be presented in the context of the development of a therapeutic strategy for the treatment of these diseases.

1. Cancer

The molecular analysis of the transport machinery represented by Rab effectors operating in the endocytic pathway is relevant to two related aspects of the molecular mechanisms underlying tumor growth: intracellular signalling and cell spreading.

Intracellular signalling

Cell growth is regulated by growth factors that by binding specific receptors on the cell surface trigger a signalling cascade that culminates with the regulation of gene expression. Growth factors and their receptors are important mitogens both in normal and in transformed cells. Mutations in their genes or overexpression can cause tumors or stimulate tumor cell growrth. For example, many tumors overexpress growth factors and surface tyrosine kinase receptors thus enhancing tumor cell growth. Growth factors and growth factor receptors have therefore been considered as excellent targets for new therapeutic drugs in the treatment of cancer and other disorders of excessive cellular proliferation. For example, the Herc2 receptor implicated in metastatic breast cancer provided the target for the development of Herceptin, a monoclonal antibody therapeutic from Genentech. The strategies pursued so far include the search for compounds that i) block ligand-receptor interactions, ii) the kinase activity of the receptors or the subsequent interactions with downstream factors of the signalling cascade. Another approach

consists of the search for drugs that modulate receptor trafficking through the endosomal system and result in receptor downregulation at the plasma membrane. Growth factor receptors and their bound ligands are internalised into endosomes, and endocytic trafficking of these molecules plays a critical role not only in attenuating the signalling response but also in establishing and controlling specific signalling pathways. The ability of growth factors to trigger signaling depends on the surface appearance of their receptors. This is determined by the kinetics and extent of receptor 1) endocytosis, 2) recycling to the cell surface and 3) transport to, and degradation in, late endocytic compartments (Sorkin et al., 1991). Molecules that can modulate these events and decrease the fraction of growth factor receptors on the plasma membrane are therefore valuable tools for an anti-cancer therapy. For example, enhanced degradation of epidermal growth factor receptor correlates with the lack of epidermal growth factor- induced proliferation and mitogen-activated protein kinase stimulation (Caraglia et al., 1999). Given their established role in the regulation of endosome structure and function, Rab GTPases and their network of Rab effectors represent an ideal target for screening of molecules that can reduce the surface content of growth factor receptors and, consequently, inhibit the growth properties of tumor cells. Rab5 for example regulates the internalisation of receptors from the plasma membrane into clathrin-coated vesicles (CCV) as well as the subsequent delivery of these carriers to early endosomes. Other Rab proteins such as Rab4 and Rab11 regulate the sorting function of early and recycling endosomes and, consequently, the recycling to the surface of growth factor receptors. The small GTPase Rab7 regulates transport of molecules from early to late endosomes and lysosomes, the degradative endocytic organelles.

The impact of Rab GTPases and their effectors/regulators on mitogenic signaling also includes the trafficking of signaling molecules downstream of growth factor receptors. An increasing number of components of the signalling machinery have recently been localised to early endocytic organelles. This remarkably specific localisation is very likely an important element in the signalling process. Such localisation depends on the structural and functional properties of the early endosomes, which in turn depends on the activity of Rab GTPases and their corresponding effectors/regulators. For example, the localisation of FYVE finger proteins depends on the synthesis of PI(3)P on the early endosome membrane (Christoforidis et al., 1999b). In the absence of this phosphoinositide these proteins are no longer capable of residing on early endosomes and are therefore released into the cytosol. Several novel signaling molecules possess a FYVE finger and, consequently, their ability to reside on early endosomes and to participate in the signaling cascade depends on the synthesis of PI(3)P. This concept is

corroborated by recent data indicating that hVPS34, the PI3-K that produces PI(3)P is required for mitogenic signaling (Siddhanta et al., 1998). The synthesis of PI(3)P is subjected to the regulation by Rab5, since PI-3 kinases (including hVPS34) are Rab5 effectors (Christoforidis et al., 1999b). Indeed, recent studies of the present inventors have demonstrated that the production of PI(3)P is stimulated by Rab5 and inhibited upon removal of Rab5 from endosomes. These observations therefore establish a direct link between the activity of Rab5 and its effectors such as PI-3 kinases and the membrane recruitment of signalling molecules to early endosomes and their ability to function in the signalling process.

Cell spreading

The growth of human tumors depends not only on the proliferative advantage characteristic of cancer cells but also on the ability of these to invade tissues and to colonise different organs. The ability of tumors to form metastatic tumours depends on the invasive properties of transformed cells. In addition, cancer cells require a higher supply of nutrients and, therefore, require de novo formation of blood vessels. Clearly, the spreading of endothelial cells during angiogenesis that occurs concomitantly with tumor growth also requires an increase in cell motility. Cell locomotion is therefore a central mechanism in tumor cell invasiveness and metastasis. On the one hand, cell spreading and invasiveness depend on the signalling function of various growth factor and growth factor receptors. In many different cell types, including tumor cells, EGFR signaling produces a pleiotropic response that includes mitogenesis or apoptosis, enhanced cell motility, protein secretion, and differentiation or dedifferentiation. The formation of new blood vessels is instead tightly regulated by specific growth factors that target receptor tyrosine kinases (RTKs) such as vascular endothelial growth factor (VEGF) (McMahon, 2000). A novel approach to treat human cancers emerging from these studies consists in the development of therapeutic strategies aimed at blocking tumor cell invasiveness and deregulated angiogenesis through the inhibition of various growth factor receptor signaling pathways (both in tumor cells and in the neighbouring tissue such as in endothelial cells). The potential use of Rab GTPases and their effectors/regulators as targets for the screening of molecules that can reduce the signaling function of growth factor receptors applies therefore not only for the mitogenic signaling but also for the spreading of tumor cells. On the other hand, the use of Rab GTPases and their effectors/regulators can also be extended to the cellular machinery that executes the signaling program resulting in increased cell locomotion. Cell locomotion occurs by a complex mechanism involving the coordinated activity of both the cytoskeleton - actin and microtubules - and membrane trafficking - especially endocytic and recycling structures. The structural/functional

properties of endosomes are important elements of the cell motility apparatus. For example, the endocytic cycle between endosomes and the plasma membrane sustains the deposition of adhesion molecules to the leading edge of motile cells. Consequently, the function of molecules that play an important role in endocytic transport can be rate-limiting important factors for the survival and spreading of cancer cells. This proposal is supported by recent studies that demonstrate that the small GTPase Rab5 is required for cell motility. The use of Rab GTPases and their effectors/regulators is therefore here applied for the screening of molecules that can reduce the ability of the cell to respond to mitogens through increased cell motility and, consequently, inhibit the spreading of tumor cells.

The described application of Rab effector intervention as a means of inhibiting or regulating cell motility defines the potential of the intervention for the treatment of other diseases that depend on cell locomotion such as endometriosis, atherosclerosis and monocyte migration into plaques, asthma and allergic diseases, and any other disease dependent on cell migration or locomotion.

2. Infectious diseases

Another area according to the present invention where membrane trafficking molecules, i.e., Rab GTPases and their effectors/regulators, can serve as therapeutic targets is that of infectious diseases. Cells internalise soluble ligands through endocytosis and large particles through phagocytosis. The latter mechanism is exploited by parasites that in this way gain access to the endomembrane system of the host cell. Increasing numbers of studies indicate that phagocytosis is regulated by the same molecular principles that govern endocytosis and recycling. For example, amphiphysin II and dynamin 2, which participate in receptor-mediated endocytosis, are also required for phagocytosis at the stage of membrane extension around the bound particles. Since the phagocytic process results in the internalisation of a large portion of the plasma membrane, mechanisms exist to ensure rapid renewal of plasma membrane. Given the role of Rab proteins in the regulation of membrane transport, it is obvious that they play an important role in the phagocytic cycle as well. Indeed, recent studies have shown that Rab11 operates in an endocytic compartment which is essential for phagocytosis. The function of endocytic Rab GTPases and their corresponding effectors/regulators are therefore expected to play an important regulatory role in the host cell invasion by parasites mediated by phagocytosis (Rab5) and in the compensatory membrane recycling (Rab4, Rab11) sustaining this process.

Once internalised, engulfed parasites or particles reside intracellularly in phagosomes. Under

normal conditions, phagosomes mature by docking and fusing progressively with early endosomes, late endosomes and lysosomes, thus causing the destruction of their content due to the low pH and increasing exposure to hydrolytic enzymes. However, it is well established that certain microbes can sabotage the cellular defence mechanisms and survive intracellularly. This is the case for example for *Mycobacterium tuberculosis* that once internalised by phagocytosis resides within the phagosome without coming in contact with harmful organelles such as lysosomes. A possible strategy to facilitate the killing of the pathogen is to re-activate the membrane trafficking route, thus resuming the maturation of the phagosome and, therefore, the exposure of the internalised bacterium to low pH and harmful hydrolytic enzymes. The strategy should focus on different Rab proteins each regulating a distinct stage of phagosome maturation (i.e. Rab5, Rab4, Rab11, Rab7).

Another area where Rab GTPases and their effectors can be used for therpeutic intervention is that of viral infection, especially HIV. AIDS continues to be cause of many deaths both in developed and under-developed countries. It has been recently established that, besides CD4, members of the family of chemokine receptors are required as co-receptors, which in conjunction with CD4, allow the virus to enter cells. Downregulation of chemokine receptors by endocytosis and inhibition of receptor recycling protects cells from HIV. These observations imply that receptor internalization and inhibition of receptor recycling represent novel therapeutic strategies to prevent HIV infection and therefore combat AIDS. Since Rab GTPases and their effectors/regulators regulate the trafficking of receptors to the cell surface, these molecules represent new targets for therapeutic agents aimed at decreasing the surface expression of HIV receptors and therefore inhibit HIV entry into cells and prevent HIV infection.

3. Diabetes

Another area where the endocytic trafficking apparatus has potential commercial applications is the trafficking of glucose transporters and the defect in this process, which is a cause of diabetes. In fat and muscle cells, stimulation by insulin causes the relocation of glucose transporters (e.g. GLUT4) from intracellular stores to the cell surface. These intracellular stores are thought to be specialised membrane domains of the recycling endomembrane system. Insulin sensitivity depends on the surface levels of such glucose transporters (GLUTs). Under physiological conditions, the increase in muscle insulin sensitivity of glucose transport observed after exercise is due to translocation of more GLUT-4 to the cell surface. Under pathological conditions, GLUTs trafficking is perturbed. Certain forms of diabetes are in fact caused by deficiency in the

surface appearance of the GLUTs. This implies that the elucidation of the transport machinery that regulates the trafficking of membrane proteins, including GLUTs, along the endocytic and recycling pathway offers therefore interesting opportunities for intervention in diabetes. In particular, the discovery of Rab effectors that play a role in the transport to endosomes and from endosomes to other destinations, e.g., the cell surface or to degradative compartments, identifies potential targets for the screening of drugs that, by altering the trafficking properties of GLUTs, can restore or stimulate their delivery to the plasma membrane in response to insulin.

4. Alzheimer's Disease

At the core of the molecular mechanism underlying the generation of Alzheimer's disease lies the aberrant processing of the Amyloid Precursor Protein (APP) by β-secretase to form Aβ(1-42). It is at present unclear where precisely the aberrant cleavage occurs, but endosomes have been implicated as the compartment or one of the compartments where processing of APP occurs. This implies that 1) APP is transported to endosomes and follows the endocytic/recycling pathway and 2) β-secretase activity is localised to endosomes. Since Rab GTPases and their effectors/regulators regulate the trafficking of molecules (lipids and proteins) in the endocytic pathway, these molecules represent new targets for therapeutic agents aimed at decreasing the presence of APP in endocytic compartments and/or the presence of β-secretase activity in endocytic compartments, therefore inhibiting the generation of beta-amyloid and retarding and or preventing the onset/progression of Alzheimer's disease.

Based on the above experimental results, the object of the present invention was to develop an assay useful for the detection and identification of molecules (pharmaceutical drugs). In order to achieve this object, the present inventors developed the strategy that molecules (proteins) interacting with GTPases such as GTPases of the Rab family and, thus, functioning at the level of endosome trafficking and coordinating the interaction of these organelles with the cytoskeleton, can provide novel targets for molecules (pharmaceutical drugs) aimed at inhibiting cell locomotion and invasiveness (and thereby cancer) as well as infectious (both viral such as AIDS and bacterial and also eukaryotic), allergic, and inflammatory diseases, diabetes, Alzheimer's disease, endometriosis, and atherosclerosis. A further object of the present invention was to provide a kit comprising molecules interacting with GTPases (effector or regulator proteins/molecules; for exact definitions see further below), which kit is useful as a means for the detection and identification of pharmaceutical drugs exhibiting properties making them preferred

drugs for treating cancer, inflammatory, allergic, and infectious diseases (eukaryotic, prokaryotic and viral) including AIDS but also atherosclerosis, endometriosis, diabetes and Alzheimer's disease. Hereinafter, these diseases are termed "the above mentioned diseases".

The following definitions are given in order to more fully specify the terms as used in the present application. **"Invasiveness"** refers to the migration and spreading of cancer cells or other cells such as endothelial cells (rather than the invasion of bacteria). **"GTPase-interacting protein"** or **"regulator/regulatory protein/regulatory molecule"** refers to any protein that interacts with the GTPase under any nucleotide conformation and for any purpose. GTPase-interacting proteins/regulators regulate the nucleotide-bound state of the GTPase, i.e., the GTPase activating protein (GAP) interacts with the GTP-bound GTPase and down regulates the activity of the GTPase by stimulating the hydrolysis of GTP. A GDP/GTP exchange factor promotes nucleotide exchange, thereby activating the GTPase. **"Effector"** or **"effector/effector protein/effector molecule"** is defined to mean a protein or a protein complex that interacts with the GTPase only if the GTPase is in the GTP-bound, also called active, form, that by binding stabilises this conformation and that mediates the "effect" of that GTPase. Effectors transmit the function of the GTPase, Rab is upstream and recruits the effectors which do the work. Regulators instead switch the Rab on and off. Rab-on (=GTP) binds the effectors and is functional. Thus, both effectors and regulators may therefore also be termed GTPase effectors and GTPase regulators, respectively. Additionally, it is apparent for the skilled reader that the term **"regulator"** is broader than the term **"effector"** and includes the latter.

The above mentioned strategy implies the first step of employing known effectors/regulators or identifying novel effectors/regulators of Rab or of other (small) GTPases, specifically regulating endosome trafficking and endosome-cytoskeleton interactions, and the additional step of designing an *in vitro* and/or *in vivo* assay to screen for inhibitors or activators of these effector/regulator molecules. In particular, the strategy includes the identification of substances useful as pharmaceutical drugs due to their interference with the process of cell locomotion, i.e., it includes the identification of inhibitors of membrane-cytoskeleton interactions.

The above object in mind, the present inventors established an assay system based on the use of such effectors/regulators, as defined hereinbefore, as targets for pharmaceutical drugs useful in the therapy of cancer and infectious diseases and any other disease which affects or requires the function of the endocytic/recycling trafficking machinery. According to a preferred embodiment

of this aspect of the present invention, an assay is provided using both an effector/regulator and a Rab protein (or any other small GTPase). Accordingly, one aspect of the present invention is the use of the above mentioned GTPase effector/regulator molecules or proteins as targets in an *in vitro* or *in vivo* assay system to detect substances useful as a pharmaceutical/therapeutic agent for the prophylaxis and the treatment of the above mentioned diseases (including cancer, allergic, inflammatory, and infectious diseases, diabetes, endometriosis, atherosclerosis and Alzheimer's disease). Another object of the present invention is a kit useful for carrying out the assay of the invention. The kit necessarily contains at least one type of effector/regulator molecule. At least one GTPase effector/regulator molecule is either native and biochemically purified from a vertebrate, or recombinant and biochemically purified from bacterial cultures, from yeast cultures, or from other cultured eukaryotic cells, in either case labelled by a covalent modification or radioactivity suitable for use in the assay.

According to a preferred embodiment of this aspect of the present invention the kit additionally contains at least one type of GTPase and/or endosomal membrane fractions obtained by subcellular fractionation and labelled by internalisation of transferrin, either fluorescently labelled or labelled by any other modification that allows its detection, and/or cytosolic extracts, and/or an ATP-regenerating system and/or a number of chemicals to be tested for their suitability as an drug against any of the above mentioned diseases (cancer, endometriosis, diabetes, atherosclerosis, allergic, infectious, inflammatory diseases). Other specific and preferred embodiments of both the kit and the use according to the present invention may be readily taken from the claims attached hereto.

Drugs exhibiting any of the above activities include those molecules (including peptides) mimicking the peptide structure of the respective target molecule. Typical representatives of the above classes are molecules/substances that carry one or more of the following functional groups: a halide atom bound to an alkyl, alkenyl, alkinyl, or aryl residue, an alcohol group (primary, secondary, tertiary), an ether group, a carbonyl function (aldehyde or ketone), a carboxylic acid group, a carboxylic anhydride group, a carbamoyl group, a haloformyl group, a cyano group, an ester group including a lactone group, a benzyl, phenyl, tolyl, tosyl, sulfonyl group, an amino group (primary, secondary, tertiary), an isocyanate, a cyanate, a thioisocyanate, a thiocyanate, a carbamate, an azide, a diazo group, and a quinone group. Other representatives of suitable drugs are organometallic compounds, sterol moiety(ies)-containing molecules, β-hydroxy carboxylic acids, inorganic acids, and complexes such as metallocenes; nucleic acids,

cytokines, hormones, antibodies, or oligopeptides comprising up to 20, preferably 8, 10, or 12, amino acid residues.

Preferably, the antibodies are polyclonal or monoclonal antibodies, or fragments thereof, humanised or even human antibodies (obtained for example by a phage display method), inhibitory or stimulatory, raised against and targeted towards any of the aforementioned GTPase effectors. A preferred nucleic acid is genomic DNA, cDNA, or mRNA, or a fragment thereof, an oligonucleotide, an oligoribonucleotide, all being based on or derived from any of the GTPase effector gene sequences having any of the sequences as depicted in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, and 15, or gene therapy vectors derived from the aforementioned GTPase effector gene sequences.

The advantage (and the gist) of the present invention consists in (1) the development of a comprehensive screening method based on molecular networks, (2) the sensitivity of the assays, and (3) the selectivity of the targets. The activity of the factors under control of the central switch can be responsible for coordinating various interconnected functions such as (a) endocytosis, (b) the distribution, i.e., sorting of proteins and lipids within endosomes and from endosomes to other intracellular locations (e.g. interactions of phagosomes with endosomes and lysosomes), (c) the motility of endosomes along the cytoskeleton, and (d) cell migration. This multiplicity of factors responsible for a wide number of cellular functions is defined as a *molecular network*. The present invention resides in enabling the skilled person to study the molecular network of many different GTPases, in particular those GTPases localised to endosomes, e.g., Rab5, Rab4, Rab11, Rab7, Rab17, Rab18, Rab22, as a whole in order to establish various (GTPase) effector/regulator proteins that are suitable as targets for pharmaceutical drugs. In this approach, the GTPase itself will not serve as a target molecule because its activity is so crucial for cell homeostasis that it would likely invariably result in toxic effects. Rather, the effector/regulator molecules specifically functioning in protein and lipid sorting, endosome motility and endosome-cytoskeleton interactions will be the target. For example, compounds that affect cell motility could be screened more specifically without affecting other important cellular functions (i.e. endocytosis). In the alternative, and according to a preferred embodiment of the present invention, the target may well be one of the effectors/regulators as defined above, but additionally a GTPase such as Rab5, Rab4, Rab7, Rab11, or Rab17, will be present in the assay system.

The present inventors have functionally characterised several Rab GTPases (e.g., Rab5, Rab4, Rab11, Rab 7, Rab17, Rab18, Rab20) and effectors/regulators of these GTPases, localised to the early endosomes and the recycling endosomes, which GTPases and effectors/regulators, respectively, are thus suitable for the assay according to the invention. These Rab proteins sequentially control transport in the endocytic and recycling pathway. Rab5 controls transport from the plasma membrane to early endosomes whereas Rab4 and Rab11 function in protein sorting and recycling from early and recycling endosomes back to the plasma membrane. The expression of Rab17 is restricted to polarised cells such as epithelial cells and neurons. This latter GTPase regulates trafficking through the apical recycling endosome, thus contributing to the generation and maintenance of cell polarity. Thus, effectors/regulators of all these GTPases and the respective GTPases (in combination with their effectors/regulators) may be employed in accordance with the present invention in an *in vitro* or *in vivo* assay system as a target in order to screen for substances that are useful as a pharmaceutical agent for the prophylaxis and treatment of the above mentioned diseases, i.e., cancer (benign tumours, malignant tumours, carcinomas, sarcomas, melanomas, leukemias, gliomas, or neuroblastomas), diabetes, Alzheimer's disease, endometriosis, atherosclerosis, allergic, inflammatory, and infectious diseases such as AIDS. tuberculosis, cholera, malaria, pseudotuberculosis, gastroenteritis, enteric fever, typhus, and any other infectious diseases caused by an infectious agent that infects cells by the endocytic route and resides intracellularly in phagosomes escaping the cellular killing mechanisms (in this regard, it should be recalled that the GTPases per se and alone can not serve as a target for the drugs to be detected; rather, the target for the drug is an effector/regulator molecule). Other examples of infectious diseases are those diseases caused by the following pathogens (bacteria or organisms): Mycobacterium, Staphylococcus, Toxoplasma, Trypanosoma, Listeria, Salmonella, Legionella, Leishmania, Coxiella, Shigella, Yersinia, Neisseria, Vibrio, Bartonella, and any other intracellular pathogen that resides in intracellular phagosomes and escapes cellular killing mechanisms.

As the proteins described herein, i.e., the Rab proteins and their effectors/regulators, are, unless exceptions, ubiquitously expressed, pharmaceutical agents for the prophylaxis and treatment of tumours of any cellular origin can be screened for and detected according to the present invention. Specifically, the following tumours may be mentioned: lung carcinoma, osteosarcoma, lymphoma, leukemia, soft tissue sarcoma, breast carcinoma, bile cancer, cervix carcinoma, cancer of the (small) intestine, of the kidneys, of the cavity of the mouth, penis carcinoma, ovary cancer, stomach cancer, cancer of the tongue, brain cancer, bladder carcinoma, prostate

carcinoma, liver carcinoma, and carcinoma of the pancreas and every tumor that invades other tissues and organs.

Drugs with anti-cancer and/or an activity against any of the other above mentioned diseases include those molecules (including peptides) mimicking the peptide structure of the respective target molecule. Typical representatives of the above classes are molecules/substances that carry one or more of the following functional groups: a halide atom bound to an alkyl, alkenyl, alkinyl or aryl residue, an alcohol group (primary, secondary, tertiary), an ether group, a carbonyl function (aldehyde or ketone), a carboxylic acid group, a carboxylic anhydride group, a carbamoyl group, a haloformyl group, a cyano group, an ester group including a lactone group, a benzyl, phenyl, tolyl, tosyl, sulfonyl group, an amino group (primary, secondary, tertiary), an isocyanate, a cyanate, a thioisocyanate, a thiocyanate, a carbamate, an azide, a diazo group, and a quinone group. Other representatives of suitable drugs are organometallic compounds, sterol moiety(ies)-containing molecules, β-hydroxy carboxylic acids, inorganic acids, and complexes such as metallocenes; nucleic acids, cytokines, hormones, antibodies, or oligopeptides comprising up to 20, preferably 8, 10, or 12, amino acid residues. Effectors transmit the function of the GTPase, Rab is upstream and recruits the effectors which do the work. Regulators instead switch the Rab on and off. Rab-on (=GTP) binds the effectors and is functional.

To elucidate the molecular mechanism whereby these GTPases exert their function in membrane trafficking and cytoskeleton organisation, the present inventors have developed a method to biochemically identify Rab5 protein effector molecules (Chistoforidis et al., 1999a). This method is based on previously described affinity chromatography (Amano et al., 1996) but has been optimised to result in the specific elution and large-scale purification of Rab effectors and GTPase interacting proteins in general, in amounts sufficient for both their identification by microsequencing techniques and their functional characterisation (Chistoforidis et al., 1999a).

In addition to that method, the present inventors have developed a novel method. This method is described in Example 3 below. With this method, the inventors have re-purified several of the proteins that bind Rab5. Using this modified affinity chromatography procedure, more than 20 effector and/or regulator proteins interacting directly or indirectly with Rab5 (Figure 1a in (Chistoforidis et al., 1999a)) have been identified and demonstrated to exhibit functional activity as shown by their ability to substitute cytosol in an *in vitro* endosome fusion reaction (Chistoforidis et al., 1999a). Some of these molecules such as Rabex-5 regulate the activity of

Rab5 (i.e., its nucleotide state) (Horiuchi et al., 1997). Others such as Rabaptin-5 and EEA1 participate in the endosome docking and fusion process (Stenmark et al., 1995). Still others regulate the ability of endosomes to move along microtubules, and all of these proteins are suitable candidates as a target for the detection of chemical drugs (compounds) that may turn out to be highly efficient agents to combat the above mentioned diseases.

In addition, expression of an activated mutant of Rab5 (Rab5Q79L) induces an increased recruitment of actin on the expanded endosomes suggesting that Rab5 either directly or indirectly regulates the attachment/nucleation of actin filaments with the endosome membrane. Based on the above data, in particular in view of this large number of Rab5 interacting molecules (more than 20), the present inventors concluded that the activity of Rab5 is not restricted to endocytic membrane docking and fusion. Most importantly, they also concluded that modulating the activity of Rab5, and therefore of its effectors/regulators, would result in changes in organelle motility *in vivo* and in cell motility. This is perfectly consistent with the effect observed by the present inventors that expression of activated Rab5 leads to an increase in motility of fibroblasts and endothelial cells, implicating this GTPase in the regulation of cell migration. The effector/regulator protein(s) responsible for this activity (to regulate membrane trafficking and membrane-cytoskeleton interactions including the ability of endosomes to move along microtubules) are therefore, in accordance with the present invention, specific targets for drugs aimed at inhibiting the ability of the Rab5 machinery to sustain cell migration.

Similar experiments have been and are being conducted on two other GTPases functioning in endosome trafficking, Rab4 and Rab11. Several candidate proteins for Rab4 effectors/regulators have been purified and one of these proteins has been sequenced and identified as the human homologue of the yeast Adenyl Cyclase-associated protein CAP (Field et al., 1990). This protein is a component of the Ras-activated cyclase complex in yeast and therefore is required for the proliferative signal in yeast. In addition, this protein has been implicated in the interaction with the actin cytoskeleton, since the carboxy terminus of CAP binds actin. Another protein which has been identified is Rabenosyn-5, a Rab5 effector. The realisation that this molecule binds both Rab5 and Rab4 but does not interact with Rab11, suggests that it may control trafficking between the Rab5 and Rab4 sub-compartments within early endosomes, and therefore regulate the sorting of proteins trafficking through this organelle. This hypothesis is consistent with the new experimental data recently obtained (see below).

Given the role of Rab proteins in the regulation of membrane transport and membrane-cytoskeleton interactions, the effectors/regulators for these small GTPases are targets for pharmaceutical drugs aimed at (1) inhibiting cell invasiveness and (2) enhancing cell-defence mechanisms against intracellular pathogens, such drugs therefore being valuable weapons in the battle against tumor (and cancer) and all other of the above mentioned diseases. For example, it may be possible to inhibit the stimulatory activity of Rab5 to reduce cell motility and consequently to reduce metastasis and the progression of cancer.

A strategy similar to that established and described above for Rab5 has been applied for other Rab proteins regulating endocytic trafficking. For example, the small GTPases Rab4, Rab11, and Rab17 reside onto and regulate trafficking through the early and recycling endosomes. Given that in motile cells recycling endosomes preferentially deliver membranes to the leading edge of the cell and given that the deposition of adhesion molecules to the leading edge of motile cells depends on the endocytic cycle between endosomes and the plasma membrane, the established functional role of Rab proteins functioning on endosomes, including Rab4 and Rab11 characterised by the present inventors, implies that they may participate in the regulation of cell motility. Therefore, the effectors/regulators of these and other endosomal GTPases will also represent novel potential targets according to the instant invention.

On the other hand, further Rab effectors/regulators to be identified and characterised in the future will also act in various aspects of endosome function, including endosome and cell motility, and endosome-phagosome interactions. An approach to identify further effectors/regulators has been described (Chistoforidis et al., 1999a), and the procedure described by the authors is similarly suitable to detect, identify, and characterise further, as yet unknown, effector/regulator molecules (proteins) of small GTPases of the Rab family.

The multiplicity of Rab5 (or any other Rab GTPases) effector/regulator molecules serves to execute distinct functions in endosome dynamics and cell dynamics all coordinated by the GTPase switch of Rab5 (or any other of the GTPases). It is therefore a "package" also referred above as a network of molecules that is necessary to coordinate different functions. These molecules can operationally be divided into the following classes on the basis of either their established identity and/or function and on the basis of their possible function for which different claims in drug discovery can be proposed. The effectors/regulators of all classes may be used for

the assay as defined herein, i.e., for the detection of suitable drugs for the treatment of any of the above mentioned diseases.

Class 1: Representative effectors/regulators: Rabaptin-5, Rabaptin-5b, Rabex-5, RN Tre, EEA1, PI 3-K, Inositol Polyphosphate 4-phosphatase and Inositol Polyphosphate 5-phosphatase.
Function: endocytic trafficking
Assay: endosome fusion, endosome motility

This class of Rab5 effectors/regulators includes molecules that 1) have either been already described as functionally linked with Rab5 (Rabaptin-5, Rabaptin-5b, Rabex-5, EEA1, PI 3-K), or 2) have previously been identified and whose DNA and deduced protein sequence have been disclosed (RN Tre, Inositol Polyphosphate 4-phosphatase and Inositol Polyphosphate 5-phosphatase) but that have been now functionally linked to Rab5 by the present inventors. Rab5 effectors/regulators of this class 1 belong to the "package" also referred above as a network of molecules that is necessary to coordinate different functions of Rab5.

Since Rab5 regulates endocytosis, effectors/regulators of this molecule also control endocytosis as well as all functions controlled by Rab5; i.e., endocytic trafficking, intracellular distribution of endosomes, motility of endosomes along microtubules, and cellular motility. Consequently, class 1 Rab5 effectors should be considered potential targets for the therapeutic strategies above described in conjunction with the other classes of Rab5 effectors/regulators.

Class 1 includes

1) Regulators that play a role in the nucleotide cycle of Rab5. Rabex-5 has been identified as a nucleotide exchange factor for Rab5 (Horiuchi et al., 1997), and therefore converts the GTPase from the inactive, GDP-bound, to the active, GTP-bound form. Consequent to the activity of Rabex-5 is the interaction between Rab5 and the Rab5 effectors that transmit Rab5 function. The present inventors have discovered that RN Tre (Matoskova et al., 1996), a protein related to the *Tre* oncogene, is a Rab5 GAP, which discovery provides an interesting link between the signal transduction and membrane trafficking machinery. Most importantly, like Rabex-5 that regulates activation of Rab5, RN Tre regulates its inactivation and therefore represents an ideal target for drugs aimed at interfering (positively and negatively) with Rab5 function.

2) Effectors that play a role in endosome docking and fusion: Rabaptin-5 (Stenmark et al., 1995), Rabaptin-5b (Gournier et al., 1998), and EEA1 (Chistoforidis et al., 1999a; Simonsen et al., 1998), or in the regulation of this process via the generation of phosphoinositide-3-phosphate (PI 3-K) which is necessary for the membrane recruitment of EEA1 (Christoforidis et al., 1999b). Two distinct phosphatidyl-inositol 3-kinases (PI 3-K), hVPS34 and p85a/p110b, have been identified among the Rab5 effectors. The two kinases are differentially distributed along the endocytic pathway, and the activity of hVPS34 is specifically required for EEA1 membrane recruitment and endosome fusion. It has also been demonstrated that VPS34 regulates the motility of endosomes along microtubules, suggesting that PI 3-K activity is necessary to coordinate the function of various Rab5 effectors/regulators, including those controlling membrane-cytoskeleton interactions (Nielsen et al., 1999). PI 3-K are therefore considered to represent a particularly suitable target of drugs that block the motility activity. Similarly, targets other than PI 3-K may be identified among the Rab5, Rab4, and Rab11 effectors/regulators that couple the activity in membrane docking and fusion with the organelle dynamics. One such target coupling the activity in membrane docking and fusion with the organelle dynamics is Rabaptin-5 due to its capacity to couple Rab4 to Rab5 function.

The present inventors have determined that the list of Rab5 effectors includes two more proteins that play a role in phosphoinositide metabolism: Inositol Polyphosphate 4-phosphatase and Inositol Polyphosphate 5-phosphatase. First tests conducted by the present inventors have proven that both these phosphatases bind to a Rab5 affinity column and directly interact with Rab5 in a yeast two-hybrid system assay. The function of Inositol Polyphosphate 4-phosphatase and Inositol Polyphosphate 5-phosphatase is to catalyse the removal of the phosphate from the 4- and 5-position of the inositol ring, respectively. In the context of the Rab5 network of effectors/regulators, these proteins are functionally linked to the PI3-Ks, especially p85a/p110b, since Inositol Polyphosphate 4-phosphatase and Inositol Polyphosphate 5-phosphatase would use as substrate the product of this kinase. The discovery that 1) Rab5 regulates a sophisticated cycle of phosphorylation/dephosphorylation of phosphoinositides and 2) that phosphoinositides regulate the recruitment of factors playing a role in the endosome transport machinery as well as signaling molecules, implies that Rab5 and its effectors/regulators can be used as drug targets for diseases where alterations in the function of endosomes can result in therapeutic intervention.

The class 1 effector/regulator molecules may be used according to the present invention as drug targets in cancer on the basis of regulating cell motility and invasiveness. The inventors of the present invention have found out that Rab5 regulates the motility of endothelial cells in vitro (this result was the outcome of experiments described in the Examples below). Since the

Rabaptin-5/Rabex-5 complexes regulate the nucleotide cycle of Rab5, and since the kinases PI 3-K regulate the recruitment of effectors/regulators, these molecules have been identified as targets for drugs aimed at inhibiting the activity of Rab5 in cell motility. The class 1 effector/regulator molecules may also be used as drug targets in cancer on the basis of the signalling molecules regulating cell growth. Growth factors and growth factor receptors are transported through the endosomes before being degraded in the late endosomes and lysosomes. Molecules that regulate the trafficking of receptors along the endocytic pathway can be used as targets for drugs aimed at interfering with transport (i.e., clearance from the plasma membrane, inhibition of recycling, transport to endosomes where signalling molecules are located, increase transport to degradative compartments where receptor-ligand complexes are degraded) and, consequently, inhibiting mitogenic signalling. Finally, the class 1 effector/regulator molecules may also be used as drug targets in infectious diseases (see the above list exemplifying infectious diseases particularly suitable according to the present invention). The small GTPase Rab5 has been observed to accumulate on *Mycobacterium tuberculosis* phagosomes. Interestingly, the Rab5 effector Rabaptin-5 is not present on the phagosome membrane. Thus, it must be concluded that there are differences between endosomes and phagosomes in the utilisation of the Rab5 effectors/regulators. In the case of *Listeria monocytogenes* phagosomes, it has been observed that an upregulation in expression of the small GTPase Rab5 occurs in the course of infection of macrophages by a *Listeria monocytogenes* non-lytic mutant. This increased Rab5 recruitment results in an increased fusogenicity of the phagosomes with endosomes leading to intracellular killing of the bacterium. Accordingly, in order to detect and identify substances suitable for the theurapeutic treatment of pathogen infections, the Rab5 effector/regulator responsible for this effect may be used as a target in the assay described in detail further below. In this case, the upregulation of the host cell defence mechanism could be accomplished by a suitable drug. The activator of Rab5, Rabex-5, that functions as a nucleotide exchange factor and converts the protein into the GTP-bound active form (Horiuchi et al., 1997) is a particularly suitable drug target in this regard. By enhancing its activity (by means of a drug activating Rabex-5), the inventors have been able to up-regulate the Rab5 machinery, stimulate endosome fusion and facilitate phagosome-endosome interactions. Alternatively, other Rab5 effectors/regulators (still to be identified) playing a role in endosome trafficking could serve as targets.

Class 1 as well as the other classes of Rab5 effectors described below may include molecules playing a role in endosome motility. The present inventors have found that Rab5 regulates the motility of early endosomes *in vivo* and both the attachment of early endosomes to microtubules

and the motility of early endosomes along microtubules *in vitro* (Nielsen et al., 1999). New data is that a Rab5 eluate is sufficient to reconstitute motility in an endosome-microtubule in vitro motility assay and in the absence of cytosol. Therefore a Rab5 effector(s) is/are sufficient to regulate or switch on a microtubule motor, or a Rab5 effector(s) is/are a microtubule motor.

For this function we make three statements to support a claim:

1. Rab5 is required to bind certain or possibly all early endosomes to microtubules.
2. Rab5 effectors and possibly their binding partners (i.e. a Rab eluate) are required for endosome motility on microtubules.
3. RabGDI blocks all endosome motility in vivo (microinjection of RabGDI).

These data demonstrate an essential role for Rab5 effectors in binding endosomes to microtubules and their subsequent motility along microtubules. Rab5 effectors can therefore be used as target molecules for drugs aiming to reduce endosome motility to block endosome cycling and, consequently, recycling of surface molecules necessary for cell motility.

Class 2

Representative effectors/regulators: Rabankyrin-5 and Rabenosyn-5 (p110 FYVE)
Function: endocytic trafficking, possible interaction with the actin cytoskeleton .
Assay: endosome fusion, endosome motility

This class includes at least two proteins which share the same PI(3)P-binding domain, the FYVE-finger. One protein is the human homologue of mouse Ankhzn (Ito et al., 1999), to be renamed Rabankyrin-5, given its ability to bind Rab5. The present inventors also found a Rabankyrin-5 splice variant (SV). Rabankyrin-5 contains ankyrin repeats. It is localised to early endosomes, binds Rab5, plays a role in endosome fusion. and is required for early endosome fusion. As regards the binding to Rab-5, it is worth noting that the present inventors determined the Rab5 binding region to reside in the ankyrin repeats in the C-terminus of the protein (between residues 542 and 1075).

The sequence of the gene coding for human Rabankyrin-5 is depicted in the sequence listing attached to this application (SEQ ID NO: 1) whereas the sequence of the protein itself is given in the sequence listing as SEQ ID NO: 2. The nucleotide and amino acid sequences of the SV gene and protein are indicated in the sequence listing as SEQ ID NO: 3 and SEQ ID NO: 4, respectively.

To the best knowledge of the present inventors, there is no prior art that would suggest a role for the gene/gene product of ankhzn - or of its human analogues - as a Rab5 effector with a role in endosome fusion and membrane trafficking. The second molecule is Rabenosyn-5, previously named p110FYVE. It is localised to early endosomes as well, binds Rab5 as well, and is also known to be complexed with and bind to the mammalian homologue of yeast Vps45p, a protein involved in transport to the vacuole. This binding demonstrates that Rabenosyn-5 has a role in SNARE-mediated membrane fusion. Rabenosyn-5 (p110FYVE) probably also plays a role in the regulation of transport and participates in the movement of endosomes along the actin and microtube cytoskeleton.

The nucleotide and amino acid sequences of p110FYVE are given in SEQ ID NO: 5 and SEQ ID NO: 6, respectively. Further properties of the effector Rabenosyn-5 (p110 FYVE) are that it is essential in endosome fusion, fusion of clathrin coated vesicles derived from the plasma membrane with early endosomes. Over-expression of Rabenosyn-5 inhibits the processing of lysosomal enzymes.

The present inventors have found that Rabenosyn-5 is a Rab4 effector. It has been known that three small GTPases, i.e., Rab5, Rab4, and Rab11, exist in separate domains on the same endosome. The present inventors have been able to demonstrate that over-expression of Rabenosyn-5 brings the Rab5 and Rab4 domains together, without changing the distribution of Rab11, and changes the sorting properties of the endosome with respect to the cycling of certain receptors (e.g. transferrin receptor). This proves that Rabenosyn-5 has a function in receptor sorting and provides validation that manipulating Rabenosyn-5 has the potential to control receptor amount, distribution, and the kinetics of receptor cycling.

Class 3

Representative effectors/regulators: multi-protein complex including p100, p95, p60, p45, p25
Function: endocytic trafficking, possibly in polarised cells such as epithelial cells and neurons
Assay: endosome fusion, endosome motility

This complex consisting of the above 5 proteins (p100, p95, p60, p45, p25) and exhibiting the molecular weights of 100 kDa, 95 kDa, 60 kDa, 45 kDa, and 25 kDa, respectively, has been isolated by the present inventors. At least one of these molecules, or the entire complex, interacts either directly with Rab5 or indirectly through another Rab5-binding protein. The present

inventors have also determined that the complex is detected in a clathrin coated vesicles-enriched fraction. This subcellular fraction includes the vesicles that transport protein receptors from the plasma membrane to the early endosomes.

The structure (sequence) and function of the 5 protein components have been investigated to some extent and are more or less known now and form part of the present invention.

In detail, the C-terminal half of the 100 kDa component shares sequence homology (52%) with the conserved catalytic domain of GTPase activating proteins and spindle assembly checkpoint proteins from yeast to mammals, while the N-terminal half shares homology (57%) with the conserved domain of serine/threonine protein kinases. Fusion between early endosomes is strongly inhibited when cytosol is immuno-depleted using anti-45 kDa component antibodies.

Immunofluorescence data show that the 45 kDa component is localised in intracellular structures which are not EEA1 positive. Upon over-expression of Rab5Q79L though, there is partial co-localisation of these proteins on early endosomes.

The nucleotide and amino acid sequences of these proteins have been determined and are depicted in the attached sequence listing (p60: SEQ ID NO: 7 and SEQ ID NO: 8; p45: SEQ ID NO: 9 and SEQ ID NO: 10; p25: SEQ ID NO: 11 and SEQ ID NO: 12, p100: SEQ ID NO: 13 and SEQ ID NO: 14; p95: SEQ ID NO: 15 and SEQ ID NO: 16, respectively). The p95 sequences are only partial sequences, however.

Examples

Example 1:

The following example describes the materials and methods used to measure the effect of a GTPase or mutant GTPase on the regulation of cell motility.

Expression constructs

Myc-rhoDV26G (Murphy et al., 1996) was cloned into the HindIII-XbaI sites of expression vector pHSVPUC (Geller et al., 1993) to generate construct: pHSV-myc-rhoDV26G. Myc-rhoDV26G cDNA was cloned in frame into the EcoRI-BamHI sites of expression vector pEGFP-C2 (Clontech) to generate a green fluorescent protein (GFP) fusion: GFP-myc-RhodV26G.

Transient transfection of rhoDV26G and human transferrin receptor

BHK-21 cells were trypsinized 24h prior to transfection and were seeded onto 11mm glass coverslips. Cells were infected with T7 RNA polymerase recombinant vaccinia virus (Bucci et al., 1992) and transfected with either T7-myc-rhoDV26G and T7-human transferrin receptor (T7-hTR) or T7-hTR alone (Murphy et al., 1996) using DOTAP (Boehringer Mannheim, Mannheim, Germany). The cell number and also the amount of DNA and lipid were constant. The final concentration of DNA per coverslip was 1μg.

Following transfection, the cells were incubated at 37°C, 5% CO_2, for 2h, cyclohexamide was then added for 1h. Cells were washed 8 times for 1min in preheated medium and rhodamine transferrin (50 μg/ml) was uptaken in a preheated humidified chamber at 37°C, 5% CO_2, for 20min. Coverslips were washed in preheated medium and then mounted into chambers for video microscopy (Bradke and Dotti, 1997; Bradke and Dotti, 1996) as described below. TLVM (time lapse video microscopy) was carried out immediately. Hydroxyurea was present at all times to prevent late viral gene expression.

Transient transfection of T7-rhoDV26G and T7-gfp

To monitor lysosomal motility in the presence and absence of rhoDV26G we made use of a green fluorescent protein-expressing vector cloned into pGEM1 harbouring the T7 promoter. Co-transfection of this plasmid with T7-myc-rhoDV26G allowed us to identify the transfected cells for video microscopy. Handling of the cells was as outlined above with the exception that they were incubated in the presence of 50 nM Lysotracker (Molecular Probes) for 15min at 37°C, 5% CO_2 to label the lysosomes, and TLVM was carried out as outlined below.

BBCE cell motility

To address the effect of rhoDV26G on cell motility, BBCE cells were trypsinized and plated at a density of 1,500 cells per cm^2, 24h later nuclear microinjection was carried out with a RhoD V26G expression construct at 50μg/ml concentration, FITC dextran was coinjected to identify the injected cells. Approximately, 12h following injection the cells were stimulated with bFGF and subjected to TLVM.

Time-Lapse Video Microscopy

The cells for TLVM were grown on coverslips and mounted into aluminum chambers as described in (Bradke and Dotti, 1997). Briefly, aluminum was cut into 7.5cm x 2.5cm x 2mm rectangles, the dimensions are similar to a standard glass slide and fit onto the microscope stage. In the centre a circular hole was cut of 8mm diameter, and around it 4mm area was milled from both sides. A glass coverslip was inserted onto one side of the chamber, sealed with a lubricant, medium was then added to fill the chamber, cells grown on coverslips were then inverted onto the other side of the metal slide and sealed with lubricant.

Image and statistical analysis of the data

A dedicated automatic program was developed to detect and track endosomes/lysosomes as they move. It runs on a SPARC station Ultra1 (SUN, Mountain View, CA) to which a Series 151/40 digital image processor (Imaging Technology, Bedford, MA) is connected. The detection of fluorescent spots corresponding to endosomes/lysosomes is performed automatically by a multiresolution algorithm based upon selectively filtering an undecimated wavelet decomposition of the image through the use of wavelet coefficient thresholding and correlation. At the end of this step, all endosomes/lysosomes in the sequence are characterised and their coordinates are determined and stored. A tracking algorithm is then used to establish valid trajectories. The algorithm uses a first-order Kalman filtering approach whereby at each frame and on the basis of the previous ones, predictions of the endosome/lysosome positions are established and compared with the computed ones. The best matches are selected as trajectory points and tracks are finally analysed to compute the data that was used for generating values in Tables 1 and 2. For each data set, a sequence of 30 images was analysed. Speed values reported here are mean values of the speed of several endosomes/lysosomes and errors are standard deviations of these group estimated.

Example 2:

Drugs that have been found by employing the assay of the present invention and that enhance or inhibit Rab5 effector/regulator activity can be distinguished and classified on the basis of their mode of action (activities 1 to 5, see below). Accordingly, 5 classes of drugs will be introduced (and searched for) that exhibit a specific mode of action on the target molecules, i.e., on the effectors or regulators:

1. Drugs that inhibit Rab5 activation. Such drugs would in principle act on the whole Rab5 network and are therefore less specific than drugs of classes 3 and 4 because they act on a

regulator (protein or molecule) that affects the nucleotide state of Rab5 and, consequently, the ability of Rab5 to signal to the totality of Rab5 effectors rather than on a specific effector (protein or molecule).

2. Drugs that enhance Rab5 inactivation. Such drugs would in principle act on the whole Rab5 network and are less specific than drugs of classes 3 and 4 for the reason given for class 1 above.

3. Drugs that disrupt specific Rab5-effector interactions, e.g., between Rab5 and p110 FYVE.

4. Drugs that inhibit a specific Rab5 effector activity, e.g., endosome fusion, endosome-microtubule interaction, endosome motility along microtubules.

5. Drugs that inhibit cell motility.

Class 3 and 4 drugs are the most specific and are therefore preferred according to the present invention. However, drugs of the other classes, in particular of classes 1 and 2, can nevertheless be considered as similarly suitable agents to combat at least one of the above mentioned diseases.

Assay for Activity 1

The assay measures Rabex-5 dependent nucleotide exchange of Rab5. The drug could perturb Rab5-Rabex-5 interaction or inhibit the catalytic activity of Rabex-5. The result expected is an inhibition of GDP/GTP exchange of Rab5. This assay is based on the assay published in (Horiuchi et al., 1997) and may well be adequately adapted for high throughput screening. It will be readily feasible, therefore, to screen thousands of compounds for their potential anti-cancer or anti-infectious disease activity/potential.

GDP/GTP exchange activity. Unless otherwise specified, the standard [^{35}S]GTPγS-binding assay was performed by the filter method (Sasaki et al., 1990) by incubating 200nM of recombinant Rab5 with 1μM of [^{35}S]GTPγS (20,000cpm/pmol) at 37°C for 10min with 5mM of $MgCl_2$ in the presence of 20nM recombinant Rabex-5.

Substances that are able to enhance or inhibit nucleotide exchange above or below the standard activity measured in the presence of Rabex-5, respectively, may be searched for.

Assay for Activity 2

The assay measures the GTPase Activating Protein (GAP) RN-Tre-dependent stimulation of GTP hydrolysis by Rab5. The drug could stabilise the Rab5-GAP interaction or stimulate the

catalytic activity of GAP. The result expected is an increase in the inactive-form of Rab5, i.e., an increased amount of GDP-bound Rab5. This assay is based on the following assay:

GAP assays with purified recombinant RN-Tre fusion proteins were performed by the filter method (Sasaki et al., 1990) using 2μM of substrate [γ-^{32}P]-GTP-loaded Rab5 in the presence of recombinant RN Tre GAP domain (100nM), at 30°C for 2min. GAP activity was expressed as the percentage of non-hydrolysed [γ-^{32}P]-GTP which remained bound to the filters, relative to the radioactivity at time 0. Substances that are able to increase the GTP hydrolysis activity compared with the standard activity measured in the presence of RN Tre should be searched for. The assay may adequately be adapted for high throughput screening.

Assay for Activity 3

Drugs may be screened for their ability to inhibit specific Rab5-Rab5 effector interactions. The assay consists in the binding of an effector molecule (either *in vitro* translated or as purified recombinant protein; native or modified, e.g., by biotinylation) to GST-Rab5:GTPgS (GTPgammaS) immobilised on a matrix. This assay is based on the procedure developed by Christoforidis et al. (1999a and b), but may be readily adapted for high throughput screening.

GST-Rab5 affinity chromatography is performed as described previously (Chistoforidis et al., 1999a). Beads with GST-Rab5 were incubated with the nucleotide exchange buffer (NE buffer) containing 20mM Hepes, 100mM NaCl, 10mM EDTA, 5mM $MgCl_2$, 1mM DTT, 1mM GTPγS, pH7.5, for 90min at room temperature under rotation. Afterwards, NE buffer was removed and the nucleotide was stabilised with NS buffer containing 20mM Hepes, 100mM NaCl, 5mM $MgCl_2$, 1mM DTT, 1mM GTPγS, pH7.5, for 20min at room temperature under rotation. *In vitro* transcription–translation of ^{35}S-methionine labeled proteins is performed according to Manufacturer's instructions (Promega). Recombinant proteins or transcribed-translated proteins (50μl of standard reactions) are incubated for 2h at 4°C with 20μl glutathione sepharose beads coupled with GST-Rab5:GTPgS, and 150μl buffer containing 20mM Hepes, 100mM NaCl, 5mM $MgCl_2$, 1mM DTT, and 1mM GTPgS, respectively, such that the final volume is 220μl. The incubation is performed both in the presence and in the absence of the candidate drug. When the drug is dissolved in an organic solvent the buffer in the control reaction will be supplemented with the solvent in the same concentration and amount added in the reaction in the presence of the drug. Usually, however, the buffer/solvent dissolving the drug is the same buffer used for the

assay, i.e., the buffer containing 20mM Hepes, 100mM NaCl, 5mM $MgCl_2$, 1mM DTT, and 1mM GTPgS. Beads are subsequently washed 4x with buffer containing 20mM Hepes, 100mM NaCl, 5mM $MgCl_2$, 1mM DTT, 10µM GTPgS, 1x with the same buffer but now containing 250mM NaCl, and 1x with 20mM Hepes, 250mM NaCl, 1mM DTT. Elution of bound proteins is performed as described before (Chistoforidis et al., 1999a; Christoforidis and Zerial, 1999c) and eluted proteins are loaded on SDS-PAGE gel followed by immunoblotting for recombinant proteins or autoradiography for *in vitro* translated proteins.

The assay measures the activity of drugs that are capable of disrupting the association of a specific or any Rab5 effector with Rab5 in a concentration-dependent manner under the same experimental conditions that allow the interaction. For example, a drug x will disrupt the interaction of effector X with Rab5:GTPgS in a concentration-dependent manner. This drug will then be used as inhibitor of effector X in the biological process X.

A variation of the assay is to use magnetic beads in an assay of the ELISA-type (similar to the *in vitro* endosome fusion assay described below). GST-Rab5 was biotinylated using chemical crosslinking (Pierce) and nucleotide exchange was performed. Beads with GST-Rab5 were incubated with NE buffer containing 20mM Hepes, 100mM NaCl, 10mM EDTA, 5mM $MgCl_2$, 1mM DTT, 1mM GTPgS, pH7.5, for 90min at room temperature under rotation. Afterwards, the NE buffer was removed and the nucleotide was stabilised with NS buffer containing 20mM Hepes, 100mM NaCl, 5mM $MgCl_2$, 1mM DTT, 1mM GTPgS, pH7.5, for 20min at room temperature under rotation. Beads (1µl) were subsequently incubated with 1µg recombinant purified Rab5 effector coupled to a ruthenium trisbipyridine chelate (IGEN) in 20µl NS buffer in the presence or absence of chemical drug candidates. Beads were then washed three times with 500µl NS buffer and binding of the effector to Rab5 is measured with an Origen Analyzer (IGEN) according to the manufacturer procedure. The background signal (binding to Rab5:GDP, i.e., without nucleotide exchange) is deducted. Drugs that affect the interaction between Rab5 and the Rab5 effector decrease the signal in comparison with the signal obtained when the reaction is performed in the absence of the drug.

The assay measures the activity of drugs that are capable of disrupting the association of a specific or any Rab5 effector with Rab5 in a concentration-dependent manner under the same experimental conditions that allow the interaction. For example, a drug x will disrupt the

interaction of effector X with Rab5:GTPgS in a concentration-dependent manner. This drug will then be used as inhibitor of effector X in the biological process X.

Assays for Activity 4

The present inventors have developed three main assays to measure Rab5 and Rab5 effector activity.

(i) *In vitro* fusion assay (Horiuchi et al., 1997)

This assay is intended to screen for drugs that inhibit endocytosis, clathrin coated vesicle (CCV) fusion with early endosomes, early endosome homotypic fusion and endosome trafficking in tumor cells or enhance endocytic membrane fusion in bacterial parasites infected cells.

Two distinct enriched populations of early endosomes labeled with either biotinylated transferrin or a sheep α-human transferrin antibody are prepared from sHeLa (suspension HeLa) cells by sucrose sedimentation (Gorvel et al., 1991). The basal fusion reaction consists of the two enriched endosome populations incubated for 45minutes at 37°C in the presence of 3mg/ml of HeLa cytosol, unlabelled transferrin and an ATP regeneration system (17.3mM creatine phosphate, 87μg/ml creatine kinase, and 2.2mM ATP) and in the presence or absence of the candidate drug. The fusion is quantified by incubation of the fusion mix with wash buffer (50mM Tris pH7.5, 100mM NaCl, 1g/l BSA and 2% (w/v) Triton X-100) and streptavidin-coated magnetic beads (Dynal). After two washes in wash buffer the samples are incubated with a rabbit α-sheep secondary antibody coupled to a ruthenium trisbipyridine chelate (IGEN) and measured with an Origen Analyzer (IGEN). The background signal (5-10% of the fusion signal) is deducted and the data expressed as the percentage of the basal fusion reaction. The CCV-early endosome fusion assay is performed in the same way with biotinylated transferrin-labeled CCV and α-transferrin antibody-labeled early endosomes.

Substances that are able to inhibit CCV-endosome or early endosome fusion below the standard fusion activity in the presence of cytosol and an ATP-regenerating system may be searched for. To control that the inhibition is targeted to a Rab5 effector (by inhibiting the Rab5-Rab5 effector interaction or the Rab5 effector activity), the inhibitory effect of the drug should be rescued by increasing concentrations of that particular Rab5 effector. For this a titration curve of the drug in the presence of increasing concentrations of the Rab5 effector will be necessary.

The following two assays are intended to screen for drugs that inhibit endosome movement and cell motility in tumor cells or enhance endocytic membrane fusion in infected cells. The assays score the overall activity, but as soon as the effector molecules specifically involved in these processes are identified, it will provide the additional possibility of using Assay for activity 3.

(ii) Microtubule spin-down assay

75 to 100μg purified early endosomes are incubated at room temperature for 20min with 100μg HeLa cytosol protein in a reaction brought to a final volume of 50μl by addition of BRB-80 (80mM K-Pipes, 1mM $MgCl_2$, 1mM EGTA, pH6.8). 10μl of taxol-stabilized microtubules (100μg tubulin equivalent) are added, and this mix is incubated for 10min at room temperature, in the presence or absence of the candidate drug, and then overlayed on 600μl of a 35% (w/v) sucrose cushion. After sedimentation at 100,000 x g for 20min at 22°C in a TLA 100.4 rotor, the upper layer was removed, the cushion was washed with 100μl BRB-80 and then also removed. The remaining pellet was resuspended in 30μl 2x SDS-PAGE buffer plus 30μl dH_2O, and analysed by SDS-PAGE and immunoblotting.

Substances that are able to inhibit the association of early endosomes with microtubules below the standard fusion activity in the presence of cytosol and an ATP-regenerating system may be searched for. To control that the inhibition is targeted to a Rab5 effector (by inhibiting the Rab5-Rab5 effector interaction or the Rab5 effector activity), the inhibitory effect of the drug may be rescued by increasing concentrations of that particular Rab5 effector. For this a titration curve of the drug in the presence of increasing concentrations of the Rab5 effector is necessary.

(iii) *In vitro* endosome motility assay

Early endosomes and cytosol are isolated as previously described (Horiuchi et al., 1997), except that HeLa spinner culture cells were allowed to internalise rhodamine-labeled transferrin for 10min at 37°C in order to label early endosomes. Oregon-green labeled tubulin is polymerized *in vitro*, and the resulting microtubules isolated, stabilised with taxol, and perfused into a microscope slide/glass coverslip chamber as previously described (Marlowe et al., 1998). The chamber is then washed with BRB-80 plus 10μM taxol to stabilise microtubules and remove non-polymerized tubulin. A mixture of HeLa cytosol (2mg/ml), MgATP (200μM), fluorescently-labeled endosomes (3mg/ml), purified bovine haemoglobin (3mg/ml; Sigma), and an anti-fade system (Howard and Hyman, 1993), in the presence or absence of the candidate drug, is perfused

into the chamber, and images of fluorescently-labeled microtubules (FITC filter set) or endosomes (Rhodamine filter set) are collected at 2sec-intervals using a time-lapse fluorescence videomicroscope. All image acquisition, processing, and analysis of movies is performed using the NIH Image v1.60 software package. Endosome movements are defined as linear, vectorial motions that occur on fluorescent microtubules over four or more consecutive images. These are discriminated from tethered Brownian motions, which generally display non-linear shaking, or flip randomly back and forth from image to image. Movements are counted from at least three and in most cases many more, individual movies, averaged and population significant differences calculated using the Excel spreadsheet program (Microsoft, Inc.). Substances that are able to inhibit the motility of early endosomes with microtubules below the standard fusion activity in the presence of cytosol and an ATP-regenerating system can be searched for. To control that the inhibition is targeted to a Rab5 effector (by inhibiting the Rab5-Rab5 effector interaction or the Rab5 effector activity), the inhibitory effect of the drug may be rescued by increasing concentrations of that particular Rab5 effector. For this a titration curve of the drug in the presence of increasing concentration of the Rab5 effector is necessary.

Assay for activity 5

This assay describes the measurement of cell motility in vitro. The assay may be standardised for high throughput screening.

To address the effect of drugs on cell motility, BBCE cells or other tumor cell lines are trypsinized and plated at a density of 1,500 cells per cm^2. 24h later the cells are stimulated with bFGF or other growth factors and subjected to Time-Lapse Video Microscopy (TLVM). The cells for TLVM are grown on coverslips and mounted into aluminum chambers as described in (Bradke and Dotti, 1997). Briefly, aluminum is cut into 7.5cm x 2.5cm x 2mm rectangles, the dimensions are similar to a standard glass slide and fit onto the microscope stage. In the centre a circular hole is cut of 8 mm diameter, and around it 4mm area is milled from both sides. A glass coverslip is inserted onto one side of the chamber, sealed with a lubricant, medium is then added to fill the chamber, cells grown on coverslips are then inverted onto the other side of the metal slide and sealed with lubricant. TLVM is carried out using a standard video microscopy set up. A dedicated automatic program was developed to detect and track fluorescently labeled cells as they move.

Example 3:

A novel Procedure for the Purification of Rab5 effectors.

120l of bacterial DH5α cells were grown to express GST-Rab5 for the large scale isolation of Rab5 effectors. The cells were induced, harvested, and broken according to manufacturer instructions (Pharmacia). Lysis buffer (2500ml) consisted of a PBS solution containing 100μM GDP, 5mM $MgCl_2$, 5mM 2-mercaptoethanol, 5μg/ml DNAase, 5μg/ml RNase, and a cocktail of protease inhibitors. High speed supernatant of the cell lysate was incubated with 20ml glutathione sepharose beads (Pharmacia) at 4°C for 2h under rotation. Subsequently, beads were loaded on a column and washed with lysis buffer without DNAase, RNase, and protease inhibitors. This procedure resulted in 1g of GST-Rab5 bound to the column. The molecule is recovered in the GDP-bound inactive form and nucleotide exchange is necessary to convert it into the GTP-bound form to allow effector binding.

The present inventors have designed an alternative procedure that overcomes the low efficiency normally affecting this step. The modification consists in the use of aluminum fluoride (AlF_3) which when bound to Ras:GDP and Mg^{2+} has been shown to mimic the transition state for GTP-hydrolysis of the GTPase complexed to GAP. The inventors have obtained evidence, however, that the method can also be applied for the complex between Rab5:GDP:Mg^{2+}:AlF_3 and its effectors. In other words, it is possible to obtain a complex consisting of Rab5, GDP, Mg^{2+}, AlF_3, and Rab5 effectors. This procedure has for example allowed the inventors to purify at large scale EEA1 and Rabankyrin-5, suggesting that this method can be applied for the identification of novel effectors and regulators for small GTPases.

Example 4:

Construction and expression of EGFP-Rab5

pEGFP-Rab5 was constructed by PCR amplification of the human Rab5a cDNA from pGEM-myc-Rab5 using the primers CCCAAGCTTATGGCTAGTCGAGGCGCAACA and AACTGCAGTTAGTTACTACAACACTGATT followed by cloning of the HindIII-PstI fragment from the PCR product into a pEGFP-C3 expression vector (Clontech, Inc.). A431 cells were grown to ~80% confluency in 10cm petri dishes and transfected with 20-30μg of plasmid DNA using a calcium phosphate based protocol. Stably transfected clonal lines were isolated after incubation in selective (G418; 0.5μg/ml) growth medium for 7-10d and checked for GFP

fluorescence on endosomal structures. Despite the presence of the selectable marker, variable levels of expression of EGFP-Rab5 in these cells were observed.

Time-lapse fluorescence videomicroscopy of EGFP-Rab5 *in vivo*

Cells were grown on glass coverslips and were transferred to custom-built aluminum microscope slide chambers (EMBL workshop, Heidelberg) just prior to observation. Unless otherwise stated, cells expressing average levels of EGFP-Rab5 were selected and analyzed on a Zeiss Axioskop microscope using 100X/1.40 plan-Apochromat lens with a temperature-controlled objective sleeve attached (EMBL workshop, Heidelberg). Time-lapse imaging was performed, collecting images at 2sec-intervals using a computer-controlled shutter (Uniblitz, Inc.) with illumination by a 100W mercury arc lamp attenuated with two heat reflection filters and a KG-1 heat absorbance filter (Zeiss). GFP-fluorescence was visualised with Hi-Q FIT, or GFP filter sets (Chroma Technologies, Inc.), and images were acquired using a COHU 4913 CCIR video camera with on-chip integration controlled by the NIH-Image v1.60 software package.

Example 5:

Microtubule spin-down assay

75 to 100µg of purified early endosomes were incubated at room temperature for 20min with 100µg HeLa cytosol protein in a reaction brought to a final volume of 50µl by addition of BRB-80 (80mM K-Pipes, 1mM $MgCl_2$, 1mM EGTA, pH6.8). 10µl of taxol-stabilized microtubules (100µg tubulin equivalent) was added and this mix was incubated for 10min at room temperature, and then overlayed on 600µl of a 35% (w/v) sucrose cushion. After sedimentation at 100,000 x g for 20min at 22°C in a TLA 100.4 rotor, the upper layer was removed, the cushion was washed with 100µl BRB-80 and then also removed. The remaining pellet was resuspended in 30µl 2x SDS-PAGE buffer plus 30µl dH_20, and analysed by SDS-PAGE and immunoblotting.

Example 6:

In vitro endosome motility assays

Early endosomes, and cytosol were isolated as previously described, except HeLa spinner culture cells were allowed to internalise rhodamine-labeled transferrin for 10min at 37°C in order to label early endosomes. Oregon-green labeled tubulin was polymerized *in vitro*, and the resulting microtubules isolated, stabilised with taxol, and perfused into a microscope slide/glass coverslip chamber as previously described. Alternatively, purified centrosomes were allowed to stick to the

glass surfaces of the chamber. Microtubule asters were then polymerized *in situ* by 30min incubation of the chamber at 37°C with fluorescently-labeled tubulin (4mg/ml) and 1mM GTP in BRB-80. The chamber was then washed with BRB-80 plus 10μM taxol to stabilise microtubules and remove non-polymerized tubulin. A mixture of HeLa cytosol (2mg/ml), MgATP (200μM), fluorescently-labeled endosomes (3mg/ml), purified bovine haemoglobin (3mg/ml; Sigma), and an anti-fade system was perfused into the chamber, and images of fluorescently-labeled microtubules (FITC filter set) or endosomes (Rhodamine filter set) were collected at 2sec-intervals using the time-lapse fluorescence videomicroscope setup described above.

Analysis and quantification of videos

All image acquisition, processing, and analysis of movies was performed using the NIH Image v1.60 software package. Endosome movements were defined as linear, vectorial motions that occurred on fluorescent microtubules over four or more consecutive images. These were discriminated from tethered Brownian motions, which generally displayed non-linear shaking, or flipped randomly back and forth from image to image. Movements were counted from at least three and in most cases many more, individual movies, averaged and population significant differences calculated using the Excel spreadsheet program (Microsoft, Inc.).

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CLAIMS

1. Use of an effector of a GTPase as a target in an *in vitro* or *in vivo* assay to detect substances useful as pharmaceutical agents for the prophylaxis and/or treatment of cancer and other proliferative, invasive or cell migration disorders such as endometriosis, atherosclerosis, inflammatory and allergic diseases, infectious diseases, diabetes, Alzheimer's disease and skin repair diseases such as psoriasis.

2. Use of claim 1, wherein the GTPase is of the Rab family.

3. Use of claim 2, wherein the GTPase is any of Rab4, Rab5, Rab7, Rab11, Rab17, Rab18, and Rab22.

4. Use of any of the preceding claims, wherein the infectious disease is AIDS, tuberculosis, pseudotuberculosis, cholera, malaria, gastroenteritis, enteric fever, typhus, those diseases caused by pathogens (bacteria or organisms) such as Mycobacterium, Staphylococcus, Toxoplasma, Trypanosoma, Listeria, Salmonella, Legionella, Leishmania, Coxiella, Shigella, Yersinia, Neisseria, Vibrio, Bartonella, or any other infectious disease caused by an infectious agent that infects cells by the endocytic route and resides intracellularly in phagosomes escaping the cellular killing mechanisms.

5. Use of any of claims 1 to 3, wherein the cancer is a benign tumor, a malignant tumor, a carcinoma, a sarcoma, a melanoma, a leukemia, a glioma, or a neuroblastoma, in particular a lung carcinoma, an osteosarcoma, a lymphoma, a soft tissue sarcoma, a breast carcinoma, a bile cancer, a cervix carcinoma, a cancer of the (small) intestine, of the kidneys, of the cavity of the mouth, a penis carcinoma, an ovary cancer, a stomach cancer, a cancer of the tongue, a brain cancer, a bladder carcinoma, a prostate carcinoma, a liver carcinoma, a carcinoma of the pancreas, and every tumor that invades other tissues and organs distinct from its site of origin.

6. Use of any of the preceding claims, wherein the assay is carried out in the presence of one or more GTPase effector/regulator molecule(s) which is/are either native and biochemically purified from a vertebrate, or recombinant and biochemically purified from

bacterial cultures, from yeast cultures, or from other cultured eukaryotic cells, in either case labeled by a covalent modification or radioactivity suitable for use in the assay.

7. Use of claim 6, wherein the assay is carried out in the simultaneous presence of at least one type of GTPase and/or endosomal membrane fractions fluorescently labeled or labeled by any other modification that allows its detection, and/or cytosolic extracts, and/or an ATP-regenerating system and/or a number of chemicals to be tested for their suitability as an anti-cancer or anti-infectious diseases drug.

8. Use of any of the preceding claims, wherein the substance useful as pharmaceutical agent is a molecule/substance that carries one or more of the following functional groups: a halide atom bound to an alkyl, alkenyl, alkinyl or aryl residue, an alcohol group (primary, secondary, tertiary), an ether group, a carbonyl function (aldehyde or ketone), a carboxylic acid group, a carboxylic anhydride group, a carbamoyl group, a haloformyl group, a cyano group, an ester group including a lactone group, a benzyl, phenyl, tolyl, tosyl, sulfonyl group, an amino group (primary, secondary, tertiary), an isocyanate, a cyanate, a thioisocyanate, a thiocyanate, a carbamate, an azide, a diazo group, and a quinone group; or is an organometallic compound, a sterol moiety(ies)-containing molecule, a β-hydroxy carboxylic acid, an inorganic acid or complex such as a metallocene, a nucleic acid, a cytokine, a hormone, an antibody, or an oligopeptide comprising up to 20, preferably 8, 10, or 12, amino acid residues.

9. Use of claim 8, wherein the antibody is a polyclonal or monoclonal antibody, or a fragment thereof, humanised or human, inhibitory or stimulatory, raised against and targeted towards any of the aforementioned GTPase effectors.

10. Use of claim 8, wherein the nucleic acid is genomic DNA, cDNA, or mRNA, or a fragment there, an oligonucleotide, an oligoribonucleotide, all being based on or derived from any of the GTPase effector having any of the sequences as depicted in SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, and 15, or gene therapy vectors derived from the aforementioned GTPase effector gene sequences.

11. A kit useful for carrying out the assay of any of the preceding claims, the kit comprising in a suitable means at least one GTPase effector/regulator molecule which is either native and biochemically purified from a vertebrate, or recombinant and biochemically purified from bacterial cultures, from yeast cultures, or from other cultured eukaryotic cells, in either case labeled by a covalent modification or by radioactivity suitable for the use in the assay.

12. The kit of claim 11, wherein the kit further comprises at least one type of GTPase and/or endosomal membrane fractions fluorescently labeled or labeled by any other modification that allows its detection, and/or cytosolic extracts, and/or an ATP-regenerating system and/or a number of chemicals to be tested for their suitability as a drug effective against any of the diseases depicted in claims 1, 4, and 5.

13. The kit of claim 12, wherein the at least one GTPase is/are one or more of the GTPases Rab4, Rab5, Rab7, Rab11, Rab17, Rab18, and Rab22.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 March 2001 (22.03.2001)

PCT

(10) International Publication Number
WO 01/20022 A1

(51) International Patent Classification[7]: C12Q 1/44

(21) International Application Number: PCT/EP00/09130

(22) International Filing Date:
18 September 2000 (18.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
99118385.6 16 September 1999 (16.09.1999) EP

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(81) Designated States *(national)*: CA, JP, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/20022 A1

(54) Title: ASSAY TO DETECT SUBSTANCES USEFUL FOR THERAPY

(57) Abstract: The present invention relates to the use of effectors/regulators for Rab and Rho GTPases in *in vitro* and *in vivo* assays that recapitulate and measure the role of these effectors/regulators in membrane transport and membrane-cytoskeleton interactions in the endocytic pathway as novel targets to find therapeutic drugs to prevent or inhibit cancer cell growth and arrest cancer cell invasiveness as well as for stimulating and/or restoring endocytic transport and phagosome maturation in cells infected by intracellular parasites, which drugs are therefore useful for the therapy and optionally also the prophylaxis of cancer and infectious diseases. In addition, the present invention is also directed to kits useful as a means to detect drugs suitable as anti-cancer and anti-infectious diseases drugs.

PATENT
DEBE:007US

DECLARATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or the below named inventors are the original, first and joint inventors (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled **ASSAY TO DETECT SUBSTANCES USEFUL FOR THERAPY**, the Specification of which:

☐ is attached hereto.
☒ was filed on **March 15, 2002** as Application Serial No. **10/088,549**.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims.

I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability of the subject matter claimed in this application, as "materiality" is defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent, United States provisional application(s), or inventor's certificate listed below and have also identified below any foreign application for patent, United States provisional application, or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIORITY APPLICATION(S)			**Priority Claimed**
EP 99118385.6	Europe	September 16, 1999	YES
(Number)	(Country)	(Date Filed)	Yes/No
(Number)	(Country)	(Date Filed)	Yes/No

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below or any PCT international application(s) designating the United States listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application or PCT international application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose all information known to me to be material to patentability of the subject matter claimed in this application, as "materiality" is defined in Title 37, Code of Federal Regulations, § 1.56,

which become available between the filing date of the prior application and the national or PCT international filing date of this application:

PCT/EP00/09130	September 18, 2000	Pending
(Application Serial No.)	(Filing Date)	(Status)
(Application Serial No.)	(Filing Date)	(Status)

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<120> ASSAY TO DETECT SUBSTANCES USEFUL FOR THERAPY

<130> DEBE:007US

<140> UNKNOWN
<141> 2002-03-15

<150> PCT/EP 00/09130
<151> 2000-09-18

<150> EP 99 118 385.6
<151> 1999-09-16

<160> 16

<170> PatentIn Ver. 2.1

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Arg Gln Glu Tyr Val Lys Leu Gln Lys Lys Leu Ala Glu Thr Glu Lys
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Arg Cys Ala Leu Leu Ala Ala Gln Ala Asn Lys Glu Ser Ser Ser Glu
        35   40   45

Ser Phe Ile Ser Arg Leu Leu Ala Ile Val Ala Asp Leu Tyr Glu Gln
    50   55   60

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Glu Gln Tyr Ser Asp Leu Lys Ile Lys Val Gly Asp Arg His Ile Ser
 65                  70                  75                  80

Ala His Lys Phe Val Leu Ala Ala Arg Ser Asp Ser Trp Ser Leu Ala
                85                  90                  95

Asn Leu Ser Ser Thr Lys Glu Leu Asp Leu Ser Asp Ala Asn Pro Glu
            100                 105                 110

Val Thr Met Thr Met Leu Arg Trp Ile Tyr Thr Asp Glu Leu Glu Phe
        115                 120                 125

Arg Glu Asp Asp Val Phe Leu Thr Glu Leu Met Lys Leu Ala Asn Arg
    130                 135                 140

Phe Gln Leu Gln Leu Leu Arg Glu Arg Cys Glu Lys Gly Val Met Ser
145                 150                 155                 160

Leu Val Asn Val Arg Asn Cys Ile Arg Phe Tyr Gln Thr Ala Glu Glu
                165                 170                 175

Leu Asn Ala Ser Thr Leu Met Asn Tyr Cys Ala Glu Ile Ile Ala Ser
            180                 185                 190

His Trp Asp Asp Leu Arg Lys Glu Asp Phe Ser Ser Met Ser Ala Gln
        195                 200                 205


Leu Leu Tyr Lys Met Ile Lys Ser Lys Thr Glu Tyr Pro Leu His Lys
    210                 215                 220

Ala Ile Lys Val Glu Arg Glu Asp Val Val Phe Leu Tyr Leu Ile Glu
225                 230                 235                 240

Met Asp Ser Gln Leu Pro Gly Lys Leu Asn Glu Ala Asp His Asn Gly
                245                 250                 255

Asp Leu Ala Leu Asp Leu Ala Leu Ser Arg Arg Leu Glu Ser Ile Ala
            260                 265                 270

Thr Thr Leu Val Ser His Lys Ala Asp Val Asp Met Val Asp Lys Ser
        275                 280                 285

Gly Trp Ser Leu Leu His Lys Gly Ile Gln Arg Gly Asp Leu Phe Ala
    290                 295                 300

Ala Thr Phe Leu Ile Lys Asn Gly Ala Phe Val Asn Ala Ala Thr Leu
305                 310                 315                 320

Gly Ala Gln Glu Thr Pro Leu His Leu Val Ala Leu Tyr Ser Ser Lys
                325                 330                 335

Lys His Ser Ala Asp Val Met Ser Glu Met Ala Gln Ile Ala Glu Ala
            340                 345                 350
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Leu Leu Gln Ala Gly Ala Asn Pro Asn Met Gln Asp Ser Lys Gly Arg
355 360 365

Thr Pro Leu His Val Ser Ile Met Ala Gly Asn Glu Tyr Val Phe Ser
370 375 380

Gln Leu Leu Gln Cys Lys Gln Leu Asp Leu Glu Leu Lys Asp His Glu
385 390 395 400

Gly Ser Thr Ala Leu Trp Leu Ala Val Gln His Ile Thr Val Ser Ser
405 410 415

Asp Gln Ser Val Asn Pro Phe Glu Asp Val Pro Val Val Asn Gly Thr
420 425 430

Ser Phe Asp Glu Asn Ser Phe Ala Ala Arg Leu Ile Gln Arg Gly Ser
435 440 445

His Thr Asp Ala Pro Asp Thr Ala Thr Gly Asn Cys Leu Leu Gln Arg
450 455 460

Ala Ala Gly Ala Gly Asn Glu Ala Ala Ala Leu Phe Leu Ala Thr Asn
465 470 475 480

Gly Ala His Val Asn His Arg Asn Lys Trp Gly Glu Thr Pro Leu His
485 490 495

Thr Ala Cys Arg His Gly Leu Ala Asn Leu Thr Ala Glu Leu Leu Gln
500 505 510

Gln Gly Ala Asn Pro Asn Leu Gln Thr Glu Glu Ala Leu Pro Leu Pro
515 520 525

Lys Glu Ala Ala Ser Leu Thr Ser Leu Ala Asp Ser Val His Leu Gln
530 535 540

Thr Pro Leu His Met Ala Ile Ala Tyr Asn His Pro Asp Val Val Ser
545 550 555 560

Val Ile Leu Glu Gln Lys Ala Asn Ala Leu His Ala Thr Asn Asn Leu
565 570 575

Gln Ile Ile Pro Asp Phe Ser Leu Lys Asp Ser Arg Asp Gln Thr Val
580 585 590

Leu Gly Leu Ala Leu Trp Thr Gly Met His Thr Ile Ala Ala Gln Leu
595 600 605

Leu Gly Ser Gly Ala Ala Ile Asn Asp Thr Met Ser Asp Gly Gln Thr
610 615 620

Leu Leu His Met Ala Ile Gln Arg Gln Asp Ser Lys Ser Ala Leu Phe
625 630 635 640

Leu Leu Glu His Gln Ala Asp Ile Asn Val Arg Thr Gln Asp Gly Glu
645 650 655

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Thr Ala Leu Gln Leu Ala Ile Arg Asn Gln Leu Pro Leu Val Val Asp
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Ala Ile Cys Thr Arg Gly Ala Asp Met Ser Val Pro Asp Glu Lys Gly
        675                 680                 685
Asn Pro Pro Leu Trp Leu Ala Leu Ala Asn Asn Leu Glu Asp Ile Ala
    690                 695                 700

Ser Thr Leu Val Arg His Gly Cys Asp Ala Thr Cys Trp Gly Pro Gly
705                 710                 715                 720

Pro Gly Gly Cys Leu Gln Thr Leu Leu His Arg Ala Ile Asp Glu Asn
                725                 730                 735

Asn Glu Pro Thr Ala Cys Phe Leu Ile Arg Ser Gly Cys Asp Val Asn
            740                 745                 750

Ser Pro Arg Gln Pro Gly Ala Asn Gly Glu Gly Glu Glu Glu Ala Arg
        755                 760                 765

Asp Gly Gln Thr Pro Leu His Leu Ala Ala Ser Trp Gly Leu Glu Glu
    770                 775                 780

Thr Val Gln Cys Leu Leu Glu Phe Gly Ala Asn Val Asn Ala Gln Asp
785                 790                 795                 800

Ala Glu Gly Arg Thr Pro Ile His Val Ala Ile Ser Ser Gln His Gly
                805                 810                 815

Val Ile Ile Gln Leu Leu Val Ser His Pro Asp Ile His Leu Asn Val
            820                 825                 830

Arg Asp Arg Gln Gly Leu Thr Pro Phe Ala Cys Ala Met Thr Phe Lys
        835                 840                 845

Asn Asn Lys Ser Ala Glu Ala Ile Leu Lys Arg Glu Ser Gly Ala Ala
    850                 855                 860

Glu Gln Val Asp Asn Lys Gly Arg Asn Phe Leu His Val Ala Val Gln
865                 870                 875                 880

Asn Ser Asp Ile Glu Ser Val Leu Phe Leu Ile Ser Val His Ala Asn
                885                 890                 895

Val Asn Ser Arg Val Gln Asp Ala Ser Lys Leu Thr Pro Leu His Leu
            900                 905                 910

Ala Val Gln Ala Gly Ser Glu Ile Ile Val Arg Asn Leu Leu Leu Ala
        915                 920                 925
Gly Ala Lys Val Asn Glu Leu Thr Lys His Arg Gln Thr Ala Leu His
    930                 935                 940

Leu Ala Ala Gln Gln Asp Leu Pro Thr Ile Cys Ser Val Leu Leu Glu
945                 950                 955                 960
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Asn Gly Val Asp Phe Ala Ala Val Asp Glu Asn Gly Asn Asn Ala Leu
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His Leu Ala Val Met His Gly Arg Leu Asn Asn Ile Arg Val Leu Leu
            980                 985                 990

Thr Glu Cys Thr Val Asp Ala Glu Ala Phe Asn Leu Arg Gly Gln Ser
        995                1000                1005

Pro Leu His Ile Leu Gly Gln Tyr Gly Lys Glu Asn Ala Ala Ala Ile
   1010                1015                1020

Phe Asp Leu Phe Leu Glu Cys Met Pro Gly Tyr Pro Leu Asp Lys Pro
1025                1030                1035                1040

Asp Ala Asp Gly Ser Thr Val Leu Leu Leu Ala Tyr Met Lys Gly Asn
                1045                1050                1055

Ala Asn Leu Cys Arg Ala Ile Val Arg Ser Gly Ala Arg Leu Gly Val
            1060                1065                1070

Asn Asn Asn Gln Gly Val Asn Ile Phe Asn Tyr Gln Val Ala Thr Lys
        1075                1080                1085

Gln Leu Leu Phe Arg Leu Leu Asp Met Leu Ser Lys Glu Pro Pro Trp
   1090                1095                1100

Cys Asp Gly Ser Tyr Cys Tyr Glu Cys Thr Ala Arg Phe Gly Val Thr
1105                1110                1115                1120

Thr Arg Lys His His Cys Arg His Cys Gly Arg Leu Leu Cys His Lys
                1125                1130                1135

Cys Ser Thr Lys Glu Ile Pro Ile Ile Lys Phe Asp Leu Asn Lys Pro
            1140                1145                1150

Val Arg Val Cys Asn Ile Cys Phe Asp Val Leu Thr Leu Gly Gly Val
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<212> PRT

<213> Homo sapiens

<400> 4

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Arg Cys Ala Leu Leu Ala Ala Gln Ala Asn Lys Glu Ser Ser Ser Glu
         35                  40                  45

Ser Phe Ile Ser Arg Leu Leu Ala Ile Val Ala Asp Leu Tyr Glu Gln
     50                  55                  60

Glu Gln Tyr Ser Ala Asn Pro Glu Val Thr Met Thr Met Leu Arg Trp
 65                  70                  75                  80
Ile Tyr Thr Asp Glu Leu Glu Phe Arg Glu Asp Asp Val Phe Leu Thr
                 85                  90                  95

Glu Leu Met Lys Leu Ala Asn Arg Phe Gln Leu Gln Leu Leu Arg Glu
            100                 105                 110

Arg Cys Glu Lys Gly Val Met Ser Leu Val Asn Val Arg Asn Cys Ile
        115                 120                 125

Arg Phe Tyr Gln Thr Ala Glu Glu Leu Asn Ala Ser Thr Leu Met Asn
    130                 135                 140

Tyr Cys Ala Glu Ile Ile Ala Ser His Trp Asp Asp Leu Arg Lys Glu
145                 150                 155                 160

Asp Phe Ser Ser Met Ser Ala Gln Leu Leu Tyr Lys Met Ile Lys Ser
                165                 170                 175

Lys Thr Glu Tyr Pro Leu His Lys Ala Ile Lys Val Glu Arg Glu Asp
            180                 185                 190

Val Val Phe Leu Tyr Leu Ile Glu Met Asp Ser Gln Leu Pro Gly Lys
        195                 200                 205

Leu Asn Glu Ala Asp His Asn Gly Asp Leu Ala Leu Asp Leu Ala Leu
    210                 215                 220

Ser Arg Arg Leu Glu Ser Ile Ala Thr Thr Leu Val Ser His Lys Ala
225                 230                 235                 240

Asp Val Asp Met Val Asp Lys Ser Gly Trp Ser Leu Leu His Lys Gly
                245                 250                 255

Ile Gln Arg Gly Asp Leu Phe Ala Ala Thr Phe Leu Ile Lys Asn Gly
            260                 265                 270

Ala Phe Val Asn Ala Ala Thr Leu Gly Ala Gln Glu Thr Pro Leu His
        275                 280                 285
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Leu Val Ala Leu Tyr Ser Ser Lys Lys His Ser Ala Asp Val Met Ser
    290                 295                 300

Glu Met Ala Gln Ile Ala Glu Ala Leu Leu Gln Ala Gly Ala Asn Pro
305                 310                 315                 320
Asn Met Gln Asp Ser Lys Gly Arg Thr Pro Leu His Val Ser Ile Met
                325                 330                 335

Ala Gly Asn Glu Tyr Val Phe Ser Gln Leu Leu Gln Cys Lys Gln Leu
            340                 345                 350

Asp Leu Glu Leu Lys Asp His Glu Gly Ser Thr Ala Leu Trp Leu Ala
        355                 360                 365

Val Gln His Ile Thr Val Ser Ser Asp Gln Ser Val Asn Pro Phe Glu
    370                 375                 380

Asp Val Pro Val Val Asn Gly Thr Ser Phe Asp Glu Asn Ser Phe Ala
385                 390                 395                 400

Ala Arg Leu Ile Gln Arg Gly Ser His Thr Asp Ala Pro Asp Thr Ala
                405                 410                 415

Thr Gly Asn Cys Leu Leu Gln Arg Ala Ala Gly Ala Gly Asn Glu Ala
            420                 425                 430

Ala Ala Leu Phe Leu Ala Thr Asn Gly Ala His Val Asn His Arg Asn
        435                 440                 445

Lys Trp Gly Glu Thr Pro Leu His Thr Ala Cys Arg His Gly Leu Ala
    450                 455                 460

Asn Leu Thr Ala Glu Leu Leu Gln Gln Gly Ala Asn Pro Asn Leu Gln
465                 470                 475                 480

Thr Glu Glu Ala Leu Pro Leu Pro Lys Glu Ala Ala Ser Leu Thr Ser
                485                 490                 495

Leu Ala Asp Ser Val His Leu Gln Thr Pro Leu His Met Ala Ile Ala
            500                 505                 510

Tyr Asn His Pro Asp Val Val Ser Val Ile Leu Glu Gln Lys Ala Asn
        515                 520                 525

Ala Leu His Ala Thr Asn Asn Leu Gln Ile Ile Pro Asp Phe Ser Leu
    530                 535                 540

Lys Asp Ser Arg Asp Gln Thr Val Leu Gly Leu Ala Leu Trp Thr Gly
545                 550                 555                 560
Met His Thr Ile Ala Ala Gln Leu Leu Gly Ser Gly Ala Ala Ile Asn
                565                 570                 575

Asp Thr Met Ser Asp Gly Gln Thr Leu Leu His Met Ala Ile Gln Arg
            580                 585                 590
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Gln Asp Ser Lys Ser Ala Leu Phe Leu Leu Glu His Gln Ala Asp Ile
595 600 605

Asn Val Arg Thr Gln Asp Gly Glu Thr Ala Leu Gln Leu Ala Ile Arg
610 615 620

Asn Gln Leu Pro Leu Val Val Asp Ala Ile Cys Thr Arg Gly Ala Asp
625 630 635 640

Met Ser Val Pro Asp Glu Lys Gly Asn Pro Pro Leu Trp Leu Ala Leu
645 650 655

Ala Asn Asn Leu Glu Asp Ile Ala Ser Thr Leu Val Arg His Gly Cys
660 665 670

Asp Ala Thr Cys Trp Gly Pro Gly Pro Gly Gly Cys Leu Gln Thr Leu
675 680 685

Leu His Arg Ala Ile Asp Glu Asn Asn Glu Pro Thr Ala Cys Phe Leu
690 695 700

Ile Arg Ser Gly Cys Asp Val Asn Ser Pro Arg Gln Pro Gly Ala Asn
705 710 715 720

Gly Glu Gly Glu Glu Glu Ala Arg Asp Gly Gln Thr Pro Leu His Leu
725 730 735

Ala Ala Ser Trp Gly Leu Glu Glu Thr Val Gln Cys Leu Leu Glu Phe
740 745 750

Gly Ala Asn Val Asn Ala Gln Asp Ala Glu Gly Arg Thr Pro Ile His
755 760 765

Val Ala Ile Ser Ser Gln His Gly Val Ile Ile Gln Leu Leu Val Ser
770 775 780

His Pro Asp Ile His Leu Asn Val Arg Asp Arg Gln Gly Leu Thr Pro
785 790 795 800

Phe Ala Cys Ala Met Thr Phe Lys Asn Asn Lys Ser Ala Glu Ala Ile
805 810 815

Leu Lys Arg Glu Ser Gly Ala Ala Glu Gln Val Asp Asn Lys Gly Arg
820 825 830

Asn Phe Leu His Val Ala Val Gln Asn Ser Asp Ile Glu Ser Val Leu
835 840 845

Phe Leu Ile Ser Val His Ala Asn Val Asn Ser Arg Val Gln Asp Ala
850 855 860

Ser Lys Leu Thr Pro Leu His Leu Ala Val Gln Ala Gly Ser Glu Ile
865 870 875 880

Ile Val Arg Asn Leu Leu Leu Ala Gly Ala Lys Val Asn Glu Leu Thr

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                885                 890                 895

Lys His Arg Gln Thr Ala Leu His Leu Ala Ala Gln Gln Asp Leu Pro
            900                 905                 910

Thr Ile Cys Ser Val Leu Leu Glu Asn Gly Val Asp Phe Ala Ala Val
        915                 920                 925

Asp Glu Asn Gly Asn Asn Ala Leu His Leu Ala Val Met His Gly Arg
    930                 935                 940

Leu Asn Asn Ile Arg Val Leu Leu Thr Glu Cys Thr Val Asp Ala Glu
945                 950                 955                 960

Ala Phe Asn Leu Arg Gly Gln Ser Pro Leu His Ile Leu Gly Gln Tyr
                965                 970                 975

Gly Lys Glu Asn Ala Ala Ala Ile Phe Asp Leu Phe Leu Glu Cys Met
            980                 985                 990

Pro Gly Tyr Pro Leu Asp Lys Pro Asp Ala Asp Gly Ser Thr Val Leu
        995                1000                1005

Leu Leu Ala Tyr Met Lys Gly Asn Ala Asn Leu Cys Arg Ala Ile Val
   1010                1015                1020

Arg Ser Gly Ala Arg Leu Gly Val Asn Asn Asn Gln Gly Val Asn Ile
1025                1030                1035                1040
Phe Asn Tyr Gln Val Ala Thr Lys Gln Leu Leu Phe Arg Leu Leu Asp
               1045                1050                1055

Met Leu Ser Lys Glu Pro Pro Trp Cys Asp Gly Ser Tyr Cys Tyr Glu
           1060                1065                1070

Cys Thr Ala Arg Phe Gly Val Thr Thr Arg Lys His His Cys Arg His
       1075                1080                1085

Cys Gly Arg Leu Leu Cys His Lys Cys Ser Thr Lys Glu Ile Pro Ile
   1090                1095                1100

Ile Lys Phe Asp Leu Asn Lys Pro Val Arg Val Cys Asn Ile Cys Phe
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<212> DNA
<213> Homo sapiens

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<210> 6
<211> 784
<212> PRT
<213> Homo sapiens

<400> 6

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Pro Leu Cys Leu Lys Asp Leu Gln Ser Phe Tyr Gln Leu His Ser His
            20                  25                  30

Tyr Glu Glu Glu His Ser Gly Glu Asp Arg Asp Val Lys Gly Gln Ile
        35                  40                  45
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Lys Ser Leu Val Gln Lys Ala Lys Lys Ala Lys Asp Arg Leu Leu Lys
50 55 60

Arg Glu Gly Asp Asp Arg Ala Glu Ser Gly Thr Gln Gly Tyr Glu Ser
65 70 75 80
Phe Ser Tyr Gly Gly Val Asp Pro Tyr Met Trp Glu Pro Gln Glu Leu
85 90 95

Gly Ala Val Arg Ser His Leu Ser Asp Phe Lys Lys His Arg Ala Ala
100 105 110

Arg Ile Asp His Tyr Val Val Glu Val Asn Lys Leu Ile Ile Arg Leu
115 120 125

Glu Lys Leu Thr Ala Phe Asp Arg Thr Asn Thr Glu Ser Ala Lys Ile
130 135 140

Arg Ala Ile Glu Lys Ser Val Val Pro Trp Val Asn Asp Gln Asp Val
145 150 155 160

Pro Phe Cys Pro Asp Cys Gly Asn Lys Phe Ser Ile Arg Asn Arg Arg
165 170 175

His His Cys Arg Leu Cys Gly Ser Ile Met Cys Lys Lys Cys Met Glu
180 185 190

Leu Ile Ser Leu Pro Leu Ala Asn Lys Leu Thr Ser Ala Ser Lys Glu
195 200 205

Ser Leu Ser Thr His Thr Ser Pro Ser Gln Ser Pro Asn Ser Val His
210 215 220

Gly Ser Arg Arg Gly Ser Ile Ser Ser Met Ser Ser Val Ser Ser Val
225 230 235 240

Leu Asp Glu Lys Asp Asp Asp Arg Ile Arg Cys Cys Thr His Cys Lys
245 250 255

Asp Thr Leu Leu Lys Arg Glu Gln Gln Ile Asp Glu Lys Glu His Thr
260 265 270

Pro Asp Ile Val Lys Leu Tyr Glu Lys Leu Arg Leu Cys Met Glu Lys
275 280 285

Val Asp Gln Lys Ala Pro Glu Tyr Ile Arg Met Ala Ala Ser Leu Asn
290 295 300

Ala Gly Glu Thr Thr Tyr Ser Leu Glu His Ala Ser Asp Leu Arg Val
305 310 315 320
Glu Val Gln Lys Val Tyr Glu Leu Ile Asp Ala Leu Ser Lys Lys Ile
325 330 335

Leu Thr Leu Gly Leu Asn Gln Asp Pro Pro Pro His Pro Ser Asn Leu
340 345 350

```
Arg Leu Gln Arg Met Ile Arg Tyr Ser Ala Thr Leu Phe Val Gln Glu
        355                 360                 365

Lys Leu Leu Gly Leu Met Ser Leu Pro Thr Lys Glu Gln Phe Glu Glu
    370                 375                 380

Leu Lys Lys Lys Arg Lys Glu Glu Met Glu Arg Lys Arg Xaa Val Glu
385                 390                 395                 400

Arg Gln Ala Ala Leu Glu Ser Gln Arg Arg Leu Glu Glu Arg Gln Ser
                405                 410                 415

Gly Leu Ala Ser Arg Ala Ala Asn Gly Glu Val Ala Ser Leu Arg Arg
            420                 425                 430

Gly Pro Ala Pro Leu Arg Lys Ala Glu Gly Trp Leu Pro Leu Ser Gly
        435                 440                 445

Gly Gln Gly Gln Ser Glu Asp Ser Asp Pro Leu Leu Gln Gln Ile His
    450                 455                 460

Asn Ile Thr Ser Phe Ile Arg Gln Ala Lys Ala Ala Gly Arg Met Asp
465                 470                 475                 480

Glu Val Arg Thr Leu Gln Glu Xaa Leu Arg Gln Leu Gln Asp Glu Tyr
                485                 490                 495

Asp Gln Gln Gln Thr Glu Lys Ala Ile Glu Leu Ser Arg Arg Gln Ala
            500                 505                 510

Glu Glu Glu Asp Leu Gln Arg Glu Gln Leu Gln Met Leu Arg Glu Arg
        515                 520                 525

Glu Leu Glu Arg Glu Arg Glu Gln Phe Arg Val Ala Ser Leu His Thr
    530                 535                 540

Arg Thr Arg Ser Leu Asp Phe Arg Glu Ile Gly Pro Phe Gln Leu Glu
545                 550                 555                 560
Pro Ser Arg Glu Pro Arg Thr His Leu Ala Tyr Ala Leu Asp Leu Gly
                565                 570                 575

Ser Ser Pro Val Pro Ser Ser Thr Ala Pro Lys Thr Pro Ser Leu Ser
            580                 585                 590

Ser Thr Gln Pro Thr Arg Val Trp Ser Gly Pro Pro Ala Val Gly Gln
        595                 600                 605

Glu Arg Leu Pro Gln Ser Ser Met Pro Gln Gln His Glu Gly Pro Ser
    610                 615                 620

Leu Asn Pro Phe Asp Glu Glu Asp Leu Ser Ser Pro Met Glu Glu Ala
625                 630                 635                 640

Thr Thr Gly Pro Pro Ala Ala Gly Val Ser Leu Asp Pro Ser Ala Arg
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645 650 655

Ile Leu Lys Glu Tyr Asn Pro Phe Glu Glu Glu Asp Glu Glu Glu Glu
660 665 670

Ala Val Ala Gly Asn Pro Phe Ile Gln Pro Asp Ser Pro Ala Pro Asn
675 680 685

Pro Phe Ser Glu Glu Asp Glu His Pro Gln Gln Arg Leu Ser Ser Pro
690 695 700

Leu Val Pro Gly Asn Pro Phe Glu Glu Pro Thr Cys Ile Asn Pro Phe
705 710 715 720

Glu Met Asp Ser Asp Ser Gly Pro Glu Ala Glu Glu Pro Ile Glu Glu
725 730 735

Glu Leu Leu Leu Gln Gln Ile Asp Asn Ile Lys Ala Tyr Ile Phe Asp
740 745 750

Ala Lys Gln Cys Gly Arg Leu Asp Glu Val Glu Val Leu Thr Glu Asn
755 760 765

Leu Arg Glu Leu Lys His Thr Leu Ala Lys Gln Lys Gly Gly Thr Asp
770 775 780

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<211> 1659
<212> DNA
<213> Homo sapiens

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agtcatttgc atggacgtct gatgctgctg cacagtttac cgtgctttat agaaaaagac 180
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tgggatgaag attttgcaga tgtgtaccat gacttaattc attctcctgc ctctgaaact 420
ctcttaaatt tggaacataa ttactttgtt agtatctcag aactgattgg tgaaagagat 480
gtggagctga aaaaattacg agagagacaa ggtattgaaa tggaaaaagt catgcaggaa 540
ttgggaaaat cactgacaga tcaagatgta aattcactgg ctgctcagca ttttgaatcc 600
cagcaagacc tagaaaataa atggtcgaat gaattaaaac aatcaactgc catccaaaaa 660
caagagtatc aagaatgggt aataaaactt caccaagacc taaaaaaccc caacaacagc 720
tcccttagtg aggaaattaa agttcagcca agtcagttca gagaatctgt agaagcaatt 780
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aagaatgctg agaggaacta tttaaatgtt ttacctgggg aatttatat tacacggcat 1260

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ttcatgatgg aaatggcttc atgggatgga ggaatttcta ggacagtgca atttctagta 1560
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<210> 8
<211> 552
<212> PRT
<213> Homo sapiens

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Tyr Lys Phe Lys Val Gly Ser Gln Cys Leu Glu Leu Arg Val Pro Leu
20 25 30

Lys Phe Pro Val Gln Glu Asn Ala Ser His Leu His Gly Arg Leu Met
35 40 45

Leu Leu His Ser Leu Pro Cys Phe Ile Glu Lys Asp Leu Lys Glu Ala
50 55 60

Leu Thr Gln Phe Ile Glu Glu Glu Ser Leu Ser Asp Tyr Asp Arg Asp
65 70 75 80

Ala Glu Ala Ser Leu Ala Ala Val Lys Ser Gly Glu Val Asp Leu His
85 90 95

Gln Leu Ala Ser Thr Trp Ala Lys Ala Tyr Ala Glu Thr Thr Leu Glu
100 105 110

His Ala Arg Pro Glu Glu Pro Ser Trp Asp Glu Asp Phe Ala Asp Val
115 120 125

Tyr His Asp Leu Ile His Ser Pro Ala Ser Glu Thr Leu Leu Asn Leu
130 135 140

Glu His Asn Tyr Phe Val Ser Ile Ser Glu Leu Ile Gly Glu Arg Asp
145 150 155 160

Val Glu Leu Lys Lys Leu Arg Glu Arg Gln Gly Ile Glu Met Glu Lys
165 170 175

Val Met Gln Glu Leu Gly Lys Ser Leu Thr Asp Gln Asp Val Asn Ser
180 185 190

Leu Ala Ala Gln His Phe Glu Ser Gln Gln Asp Leu Glu Asn Lys Trp
195 200 205

Ser Asn Glu Leu Lys Gln Ser Thr Ala Ile Gln Lys Gln Glu Tyr Gln
210 215 220

```
Glu Trp Val Ile Lys Leu His Gln Asp Leu Lys Asn Pro Asn Asn Ser
225                 230                 235                 240

Ser Leu Ser Glu Glu Ile Lys Val Gln Pro Ser Gln Phe Arg Glu Ser
                245                 250                 255
Val Glu Ala Ile Gly Arg Ile Tyr Glu Glu Gln Arg Lys Leu Glu Glu
            260                 265                 270

Ser Phe Thr Ile His Leu Gly Ala Gln Leu Lys Thr Met His Asn Leu
        275                 280                 285

Arg Leu Leu Arg Ala Asp Met Leu Asp Phe Cys Lys His Lys Arg Asn
    290                 295                 300

His Arg Ser Gly Val Lys Leu His Arg Leu Gln Thr Ala Leu Ser Leu
305                 310                 315                 320

Tyr Ser Thr Ser Leu Cys Gly Leu Val Leu Leu Val Asp Asn Arg Ile
                325                 330                 335

Asn Ser Tyr Ser Gly Ile Lys Arg Asp Phe Ala Thr Val Cys Gln Glu
            340                 345                 350

Cys Thr Asp Phe His Phe Pro Arg Ile Glu Glu Gln Leu Glu Val Val
        355                 360                 365

Gln Gln Val Val Leu Tyr Ala Arg Thr Gln Arg Arg Ser Lys Leu Lys
    370                 375                 380

Glu Ser Leu Asp Ser Gly Asn Gln Asn Gly Gly Asn Asp Asp Lys Thr
385                 390                 395                 400

Lys Asn Ala Glu Arg Asn Tyr Leu Asn Val Leu Pro Gly Glu Phe Tyr
                405                 410                 415

Ile Thr Arg His Ser Asn Leu Ser Glu Ile His Val Ala Phe His Leu
            420                 425                 430

Cys Val Asp Asp His Val Lys Ser Gly Asn Ile Thr Ala Arg Asp Pro
        435                 440                 445

Ala Ile Met Gly Leu Arg Asn Ile Leu Lys Val Cys Cys Thr His Asp
    450                 455                 460

Ile Thr Thr Ile Ser Ile Pro Leu Leu Leu Val His Asp Met Ser Glu
465                 470                 475                 480

Glu Met Thr Ile Pro Trp Cys Leu Arg Arg Ala Glu Leu Val Phe Lys
                485                 490                 495
Cys Val Lys Gly Phe Met Met Glu Met Ala Ser Trp Asp Gly Gly Ile
            500                 505                 510

Ser Arg Thr Val Gln Phe Leu Val Pro Gln Ser Ile Ser Glu Glu Met
        515                 520                 525
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Phe Tyr Gln Leu Ser Asn Met Leu Pro Gln Ile Phe Arg Val Ser Ser
    530                 535                 540

Thr Leu Thr Leu Thr Ser Lys His
545                 550
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<211> 1050
<212> DNA
<213> Homo sapiens

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gctctgagcc agataaatac aaagcttctg gcagaaatga agatgaaaaa ggatttattt 180
cctgttggga gagaaattgc tggaattgta ttagatgttg gaagcaaggt atcattcttt 240
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ggaagaaaaa agcaagttgt tcaattttaa 1050

<210> 10
<211> 349
<212> PRT
<213> Homo sapiens

<400> 10
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Met Lys Gly Leu Tyr Phe Gln Gln Ser Ser Thr Asp Glu Glu Ile Thr
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Phe Val Phe Gln Glu Lys Glu Asp Leu Pro Val Thr Glu Asp Asn Phe
             20                  25                  30

Val Lys Leu Gln Val Lys Ala Cys Ala Leu Ser Gln Ile Asn Thr Lys
         35                  40                  45

Leu Leu Ala Glu Met Lys Met Lys Lys Asp Leu Phe Pro Val Gly Arg
     50                  55                  60

Glu Ile Ala Gly Ile Val Leu Asp Val Gly Ser Lys Val Ser Phe Phe
 65                  70                  75                  80
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Gln Pro Asp Asp Glu Val Val Gly Ile Leu Pro Leu Asp Ser Glu Asp
                 85                  90                  95

Pro Gly Leu Cys Glu Val Val Arg Val His Glu His Tyr Leu Val His
            100                 105                 110

Lys Pro Glu Lys Val Thr Trp Thr Glu Ala Ala Gly Ser Ile Arg Asp
        115                 120                 125

Gly Val Arg Ala Tyr Thr Ala Leu His Tyr Leu Ser His Leu Ser Pro
    130                 135                 140

Gly Lys Ser Val Leu Ile Met Asp Gly Ala Ser Ala Phe Gly Thr Ile
145                 150                 155                 160

Ala Ile Gln Leu Ala His His Arg Gly Ala Lys Val Ile Ser Thr Ala
                165                 170                 175

Cys Ser Leu Glu Asp Lys Gln Cys Leu Glu Arg Phe Arg Pro Pro Ile
            180                 185                 190

Ala Arg Val Ile Asp Val Ser Asn Gly Lys Val His Val Ala Glu Ser
        195                 200                 205

Cys Leu Glu Glu Thr Gly Gly Leu Gly Val Asp Ile Val Leu Asp Ala
    210                 215                 220


Gly Val Arg Leu Tyr Ser Lys Asp Asp Glu Pro Ala Val Lys Leu Gln
225                 230                 235                 240

Leu Leu Pro His Lys His Asp Ile Ile Thr Leu Leu Gly Val Gly Gly
                245                 250                 255

His Trp Val Thr Thr Glu Glu Asn Leu Gln Leu Asp Pro Pro Asp Ser
            260                 265                 270

His Cys Leu Phe Leu Lys Gly Ala Thr Leu Ala Phe Leu Asn Asp Glu
        275                 280                 285

Val Trp Asn Leu Ser Asn Val Gln Gln Gly Lys Tyr Leu Cys Ile Leu
    290                 295                 300

Lys Asp Val Met Glu Lys Leu Ser Thr Gly Val Phe Arg Pro Gln Leu
305                 310                 315                 320

Asp Glu Pro Ile Pro Leu Tyr Glu Ala Lys Val Ser Met Glu Ala Val
                325                 330                 335

Gln Lys Asn Gln Gly Arg Lys Lys Gln Val Val Gln Phe
            340                 345
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<211> 663

<212> DNA
<213> Homo sapiens

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tga 663

<210> 12
<211> 220
<212> PRT
<213> Homo sapiens

<400> 12
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Ser Gly Ala Ala Glu Gly Val Ser Ala Gln Ser Phe Leu His Cys Phe
20 25 30

Thr Met Ala Ser Thr Ala Phe Asn Leu Gln Val Ala Thr Pro Gly Gly
35 40 45

Lys Ala Met Glu Phe Val Asp Val Thr Glu Ser Asn Ala Arg Trp Val
50 55 60

Gln Asp Phe Arg Leu Lys Ala Tyr Ala Ser Pro Ala Lys Leu Glu Ser
65 70 75 80

Ile Asp Gly Ala Arg Tyr His Ala Leu Leu Ile Pro Ser Cys Pro Gly
85 90 95

Ala Leu Thr Asp Leu Ala Ser Ser Gly Ser Leu Ala Arg Ile Leu Gln
100 105 110

His Phe His Ser Glu Ser Lys Pro Ile Cys Ala Val Gly His Gly Val
115 120 125

Ala Ala Leu Cys Cys Ala Thr Asn Glu Asp Arg Ser Trp Val Phe Asp
130 135 140

Ser Tyr Ser Leu Thr Gly Pro Ser Val Cys Glu Leu Val Arg Ala Pro
145 150 155 160

Gly Phe Ala Arg Leu Pro Leu Val Val Glu Asp Phe Val Lys Asp Ser
165 170 175

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Gly Ala Cys Phe Ser Gly Leu Gly Ala Ala Pro Gly Trp Gly Arg Gly
            180                 185                 190

Gly Lys Gln Glu Asp Arg Pro Pro Glu Glu Gln Arg Arg Ala Gly Glu
        195                 200                 205
Asp Phe Pro Ile Tyr Gln Cys Val Phe Ile Tyr Pro
    210                 215                 220
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<210> 13
<211> 2679
<212> DNA
<213> Homo sapiens

<400> 13

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Thr His Pro Arg Leu Cys Gln Tyr Val Asp Ile Ser Arg Gly Lys His
50 55 60

Glu Arg Leu Val Val Val Ala Glu His Cys Glu Arg Ser Leu Glu Asp
65 70 75 80

Leu Leu Arg Glu Arg Lys Pro Val Ser Cys Ser Thr Val Leu Cys Ile
85 90 95

Ala Phe Glu Val Leu Gln Gly Leu Gln Tyr Met Asn Lys His Gly Ile
100 105 110

Val His Arg Ala Leu Ser Pro His Asn Ile Leu Leu Asp Arg Lys Gly
115 120 125

His Ile Lys Leu Ala Lys Phe Gly Leu Tyr His Met Thr Ala His Gly
130 135 140

Asp Asp Val Asp Phe Pro Ile Gly Tyr Pro Ser Tyr Leu Ala Pro Glu
145 150 155 160

Val Ile Ala Gln Gly Ile Phe Lys Thr Thr Asp His Met Pro Ser Lys
165 170 175

Lys Pro Leu Pro Ser Gly Pro Lys Ser Asp Val Trp Ser Leu Gly Ile
180 185 190

Ile Leu Phe Glu Leu Cys Val Gly Arg Lys Leu Phe Gln Ser Leu Asp
195 200 205

Ile Ser Glu Arg Leu Lys Phe Leu Leu Thr Leu Asp Cys Val Asp Asp

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Glu Leu Pro Glu Thr Val Ile Asp Leu Leu Asn Lys Cys Leu Thr Phe
                245                 250                 255

His Pro Ser Lys Arg Pro Thr Pro Asp Glu Leu Met Lys Asp Lys Val
            260                 265                 270

Phe Ser Glu Val Ser Pro Leu Tyr Thr Pro Phe Thr Lys Pro Ala Ser
        275                 280                 285

Leu Phe Ser Ser Ser Leu Arg Cys Ala Asp Leu Thr Leu Pro Glu Asp
    290                 295                 300

Ile Ser Gln Leu Cys Lys Asp Ile Asn Asn Asp Tyr Leu Ala Glu Arg
305                 310                 315                 320

Ser Ile Glu Glu Val Tyr Tyr Leu Trp Cys Leu Ala Gly Gly Asp Leu
                325                 330                 335

Glu Lys Glu Leu Val Asn Lys Glu Ile Ile Arg Ser Lys Pro Pro Ile
            340                 345                 350

Cys Thr Leu Pro Asn Phe Leu Phe Glu Asp Gly Glu Ser Phe Gly Gln
        355                 360                 365
Gly Arg Asp Arg Ser Ser Leu Leu Asp Asp Thr Thr Val Thr Leu Ser
    370                 375                 380

Leu Cys Gln Leu Arg Asn Arg Leu Lys Asp Val Gly Gly Glu Ala Phe
385                 390                 395                 400

Tyr Pro Leu Leu Glu Asp Asp Gln Ser Asn Leu Pro His Ser Asn Ser
                405                 410                 415

Asn Asn Glu Leu Ser Ala Ala Ala Met Leu Pro Leu Ile Ile Arg Glu
            420                 425                 430

Lys Asp Thr Glu Tyr Gln Leu Asn Arg Ile Ile Leu Phe Asp Arg Leu
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Lys Ala Tyr Pro Tyr Lys Lys Asn Gln Ile Trp Lys Glu Ala Arg Val
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Asp Ile Pro Pro Leu Met Arg Gly Leu Thr Trp Ala Ala Leu Leu Gly
465                 470                 475                 480

Val Glu Gly Ala Ile His Ala Lys Tyr Asp Ala Ile Asp Lys Asp Thr
                485                 490                 495

Pro Ile Pro Thr Asp Arg Gln Ile Glu Val Asp Ile Pro Arg Cys His
            500                 505                 510
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Gln Tyr Asp Glu Leu Leu Ser Ser Pro Glu Gly His Ala Lys Phe Arg
        515                 520                 525

Arg Val Leu Lys Ala Trp Val Val Ser His Pro Asp Leu Val Tyr Trp
    530                 535                 540

Gln Gly Leu Asp Ser Leu Cys Ala Pro Phe Leu Tyr Leu Asn Phe Asn
545                 550                 555                 560

Asn Glu Ala Leu Ala Tyr Ala Cys Met Ser Ala Phe Ile Pro Lys Tyr
                565                 570                 575

Leu Tyr Asn Phe Phe Leu Lys Asp Asn Ser His Val Ile Gln Glu Tyr
            580                 585                 590

Leu Thr Val Phe Ser Gln Met Ile Ala Phe His Asp Pro Glu Leu Ser
        595                 600                 605
Asn His Leu Asn Gln Ile Gly Phe Ile Pro Asp Leu Tyr Ala Ile Pro
    610                 615                 620

Trp Phe Leu Thr Met Phe Thr His Val Phe Pro Leu His Lys Ile Phe
625                 630                 635                 640

His Leu Trp Asp Thr Leu Leu Leu Gly Asn Ser Ser Phe Pro Phe Cys
                645                 650                 655

Ile Gly Val Ala Ile Leu Gln Gln Leu Arg Asp Arg Leu Leu Ala Asn
            660                 665                 670

Gly Phe Asn Glu Cys Ile Leu Leu Phe Ser Asp Leu Pro Glu Ile Asp
        675                 680                 685

Ile Glu Arg Cys Val Arg Glu Ser Ile Asn Leu Phe Cys Trp Thr Pro
    690                 695                 700

Lys Ser Ala Thr Tyr Arg Gln His Ala Gln Pro Pro Lys Pro Ser Ser
705                 710                 715                 720

Asp Ser Ser Gly Gly Arg Ser Ser Ala Pro Tyr Phe Ser Ala Glu Cys
                725                 730                 735

Pro Asp Pro Pro Lys Thr Asp Leu Ser Arg Glu Ser Ile Pro Leu Asn
            740                 745                 750

Asp Leu Lys Ser Glu Val Ser Pro Arg Ile Ser Ala Glu Asp Leu Ile
        755                 760                 765

Asp Leu Cys Glu Leu Thr Val Thr Gly His Phe Lys Thr Pro Ser Lys
    770                 775                 780

Lys Thr Lys Ser Ser Lys Pro Lys Leu Leu Val Val Asp Ile Leu Asn
785                 790                 795                 800

Ser Glu Asp Phe Ile Arg Gly His Ile Ser Gly Ser Ile Asn Ile Pro
                805                 810                 815
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Phe Ser Ala Ala Phe Thr Ala Glu Gly Glu Leu Thr Gln Gly Pro Tyr
            820   825   830

Thr Ala Met Leu Gln Asn Phe Lys Gly Lys Val Ile Val Ile Val Gly
        835   840   845
His Val Ala Lys His Thr Ala Glu Phe Ala Ala His Leu Val Lys Met
    850   855   860

Lys Tyr Pro Arg Ile Cys Ile Leu Asp Gly Gly Ile Asn Lys Ile Lys
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Pro Thr Gly Leu Leu Thr Ile Pro Ser Pro Gln Ile
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<213> Homo sapiens

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gctctcaatg ttcccctgca caataggcga caccagctga agatgcgaga tatagctggg 180
caggccctgg cttttgttca ggatcttgta ccggctcttc tgaactttca cacctataca 240
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<212> PRT
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             20                  25                  30

Asp Thr Lys Cys His Leu Tyr Asn Ala Leu Asn Val Pro Leu His Asn
         35                  40                  45

Arg Arg His Gln Leu Lys Met Arg Asp Ile Ala Gly Gln Ala Leu Ala
     50                  55                  60

Phe Val Gln Asp Leu Val Pro Ala Leu Leu Asn Phe His Thr Tyr Thr
 65                  70                  75                  80

Glu Gln Arg Ile Gln Ile Phe Pro Val Asp Ser Ala Ile Asp Thr Ile
                 85                  90                  95

Ser Pro Leu Asn Gln Lys Phe Ser Gln Tyr Leu His Glu Asn Ala Ser
            100                 105                 110

Tyr Val Arg Pro Leu Glu Glu Gly Met Leu His Leu Phe Glu Ser Ile
        115                 120                 125

Thr Glu Asp Thr Val Thr Val Leu Glu Thr Thr Val Lys Leu Lys Met
    130                 135                 140

Phe Ser Asp His Leu Thr Ser Tyr Val Arg Phe Leu Arg Lys Ile Leu
145                 150                 155                 160

Pro Tyr Gln Leu Lys Ser Leu Glu Glu Glu Cys Glu Ser Ser Leu Cys
                165                 170                 175

Thr Pro Ala Leu Arg Ala Arg Asn Leu Glu Leu Ser Gln Asp Met Lys
            180                 185                 190

Thr Met Thr Ala Val Phe Glu Lys Leu Gln Thr Tyr Val Thr Leu Leu
        195                 200                 205

Ala Leu Pro Ser Thr Glu Pro Asp Gly Leu Leu Arg Thr Asn Tyr Thr
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Ser Val Leu Thr Asn Val Gly Ala Ala Leu His Gly Phe His Asp Val
225                 230                 235                 240

Met Lys Asp Ile Ser Lys His Tyr Ser Gln Lys Ala Ser Ile Glu His
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Glu Ile Pro Thr Ala Thr Gln Lys Leu Val Thr Thr Asn Asp Cys Ile
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Leu Ser Ser Ala Val Thr Leu Thr Asn Gly Ala Gly Lys Ile Ala Ser
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Phe Phe Gly Asn Asn Val Asp Tyr Phe Ile Ala Ser Leu Ser Tyr Gly
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Pro Lys Thr Ala Ser Gly Phe Ile Ser Pro Leu Ser Ala Glu Cys Met
305                 310                 315                 320

Leu Gln Tyr Lys Lys Lys Ala Ala Ala Tyr Met Lys Ser Leu Arg Thr
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Pro Leu Ala Glu Ser Val Pro Tyr Gly Glu Ala Val Ala Asn Arg Arg
            340                 345                 350

Val Leu Leu Ser Ser Thr Glu Ser Arg Glu Gly Leu Ala Gln Gln Val
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Gln Gln Ser Leu Glu Lys Ile Ser Lys Leu Glu Gln Glu Lys Glu His
    370                 375                 380

Trp Met Leu Glu Ala Gln Leu Ala Lys Ile Lys Leu Glu Lys Glu Asn
385                 390                 395                 400

Gln Arg Ile Ala Asp Arg Leu Arg Gly Thr Thr Ser Ala Gln Leu Pro
                405                 410                 415
Gly Leu Ala Gln Glu Asn Ala Thr Val Pro Ile Ala Ser Ser Gln Glu
            420                 425                 430

Glu Ala Ala Ala Lys Val Leu Thr Glu Pro Val Gln Ser Thr Ser Leu
        435                 440                 445

Val Gly Met Leu Thr Arg Thr Pro Asp Ser Glu Ala Pro Asp Val Glu
    450                 455                 460

Ser Arg Glu Asp Leu Ile Lys Ser His Tyr Met Ala Arg Ile Ala Glu
465                 470                 475                 480

Leu Thr Ser Gln Leu Gln Leu Ala Asp Ser Lys Ser Val His Phe Tyr
                485                 490                 495

Ala Glu Cys Arg Ala Leu Ser Lys Arg Leu Ala Leu Ala Glu Lys Ser
            500                 505                 510

Lys Glu Thr Leu Thr Glu Glu Met Arg Leu Ala Ser Gln Asn Ile Ser
        515                 520                 525

Arg Leu Gln Asp Glu Leu Met Thr Thr Lys Arg Ser Tyr Glu Asp Gln
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Leu Ser Met Met Ser Asp His Leu Cys Ser Met Asn Glu Thr Leu Ser
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Ser Lys Lys Thr Arg Asn Arg
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SEQUENCE LISTING

<110> Erik Nielsen
Savvas Chritophoridis
Carol Murphy
Marino Zerial
Stefano De Renzis

<120> ASSAY TO DETECT SUBSTANCES USEFUL FOR THERAPY

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<150> EP 99 118 385.6
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<212> PRT
<213> Homo sapiens

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Arg Cys Ala Leu Leu Ala Ala Gln Ala Asn Lys Glu Ser Ser Ser Glu
         35                  40                  45

Ser Phe Ile Ser Arg Leu Leu Ala Ile Val Ala Asp Leu Tyr Glu Gln
     50                  55                  60
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Glu Gln Tyr Ser Asp Leu Lys Ile Lys Val Gly Asp Arg His Ile Ser
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Ala His Lys Phe Val Leu Ala Ala Arg Ser Asp Ser Trp Ser Leu Ala
85 90 95

Asn Leu Ser Ser Thr Lys Glu Leu Asp Leu Ser Asp Ala Asn Pro Glu
100 105 110

Val Thr Met Thr Met Leu Arg Trp Ile Tyr Thr Asp Glu Leu Glu Phe
115 120 125

Arg Glu Asp Asp Val Phe Leu Thr Glu Leu Met Lys Leu Ala Asn Arg
130 135 140

Phe Gln Leu Gln Leu Leu Arg Glu Arg Cys Glu Lys Gly Val Met Ser
145 150 155 160

Leu Val Asn Val Arg Asn Cys Ile Arg Phe Tyr Gln Thr Ala Glu Glu
165 170 175

Leu Asn Ala Ser Thr Leu Met Asn Tyr Cys Ala Glu Ile Ile Ala Ser
180 185 190

His Trp Asp Asp Leu Arg Lys Glu Asp Phe Ser Ser Met Ser Ala Gln
195 200 205

Leu Leu Tyr Lys Met Ile Lys Ser Lys Thr Glu Tyr Pro Leu His Lys
210 215 220

Ala Ile Lys Val Glu Arg Glu Asp Val Val Phe Leu Tyr Leu Ile Glu
225 230 235 240

Met Asp Ser Gln Leu Pro Gly Lys Leu Asn Glu Ala Asp His Asn Gly
245 250 255

Asp Leu Ala Leu Asp Leu Ala Leu Ser Arg Arg Leu Glu Ser Ile Ala
260 265 270

Thr Thr Leu Val Ser His Lys Ala Asp Val Asp Met Val Asp Lys Ser
275 280 285

Gly Trp Ser Leu Leu His Lys Gly Ile Gln Arg Gly Asp Leu Phe Ala
290 295 300

Ala Thr Phe Leu Ile Lys Asn Gly Ala Phe Val Asn Ala Ala Thr Leu
305 310 315 320

Gly Ala Gln Glu Thr Pro Leu His Leu Val Ala Leu Tyr Ser Ser Lys
325 330 335

Lys His Ser Ala Asp Val Met Ser Glu Met Ala Gln Ile Ala Glu Ala
340 345 350

Leu Leu Gln Ala Gly Ala Asn Pro Asn Met Gln Asp Ser Lys Gly Arg
355 360 365

Thr Pro Leu His Val Ser Ile Met Ala Gly Asn Glu Tyr Val Phe Ser
370 375 380

Gln Leu Leu Gln Cys Lys Gln Leu Asp Leu Glu Leu Lys Asp His Glu
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Gly Ser Thr Ala Leu Trp Leu Ala Val Gln His Ile Thr Val Ser Ser
405 410 415

Asp Gln Ser Val Asn Pro Phe Glu Asp Val Pro Val Val Asn Gly Thr
420 425 430

Ser Phe Asp Glu Asn Ser Phe Ala Ala Arg Leu Ile Gln Arg Gly Ser
435 440 445

His Thr Asp Ala Pro Asp Thr Ala Thr Gly Asn Cys Leu Leu Gln Arg
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Ala Ala Gly Ala Gly Asn Glu Ala Ala Ala Leu Phe Leu Ala Thr Asn
465 470 475 480

Gly Ala His Val Asn His Arg Asn Lys Trp Gly Glu Thr Pro Leu His
485 490 495

Thr Ala Cys Arg His Gly Leu Ala Asn Leu Thr Ala Glu Leu Leu Gln
500 505 510

Gln Gly Ala Asn Pro Asn Leu Gln Thr Glu Glu Ala Leu Pro Leu Pro
515 520 525

Lys Glu Ala Ala Ser Leu Thr Ser Leu Ala Asp Ser Val His Leu Gln
530 535 540

Thr Pro Leu His Met Ala Ile Ala Tyr Asn His Pro Asp Val Val Ser
545 550 555 560

Val Ile Leu Glu Gln Lys Ala Asn Ala Leu His Ala Thr Asn Asn Leu
565 570 575

Gln Ile Ile Pro Asp Phe Ser Leu Lys Asp Ser Arg Asp Gln Thr Val
580 585 590

Leu Gly Leu Ala Leu Trp Thr Gly Met His Thr Ile Ala Ala Gln Leu
595 600 605

Leu Gly Ser Gly Ala Ala Ile Asn Asp Thr Met Ser Asp Gly Gln Thr
610 615 620

Leu Leu His Met Ala Ile Gln Arg Gln Asp Ser Lys Ser Ala Leu Phe
625 630 635 640

Leu Leu Glu His Gln Ala Asp Ile Asn Val Arg Thr Gln Asp Gly Glu
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660 665 670

Ala Ile Cys Thr Arg Gly Ala Asp Met Ser Val Pro Asp Glu Lys Gly
675 680 685

Asn Pro Pro Leu Trp Leu Ala Leu Ala Asn Asn Leu Glu Asp Ile Ala
690 695 700

Ser Thr Leu Val Arg His Gly Cys Asp Ala Thr Cys Trp Gly Pro Gly
705 710 715 720

Pro Gly Gly Cys Leu Gln Thr Leu Leu His Arg Ala Ile Asp Glu Asn
725 730 735

Asn Glu Pro Thr Ala Cys Phe Leu Ile Arg Ser Gly Cys Asp Val Asn
740 745 750

Ser Pro Arg Gln Pro Gly Ala Asn Gly Glu Gly Glu Glu Glu Ala Arg
755 760 765

Asp Gly Gln Thr Pro Leu His Leu Ala Ala Ser Trp Gly Leu Glu Glu
770 775 780

Thr Val Gln Cys Leu Leu Glu Phe Gly Ala Asn Val Asn Ala Gln Asp
785 790 795 800

Ala Glu Gly Arg Thr Pro Ile His Val Ala Ile Ser Ser Gln His Gly
805 810 815

Val Ile Ile Gln Leu Leu Val Ser His Pro Asp Ile His Leu Asn Val
820 825 830

Arg Asp Arg Gln Gly Leu Thr Pro Phe Ala Cys Ala Met Thr Phe Lys
835 840 845

Asn Asn Lys Ser Ala Glu Ala Ile Leu Lys Arg Glu Ser Gly Ala Ala
850 855 860

Glu Gln Val Asp Asn Lys Gly Arg Asn Phe Leu His Val Ala Val Gln
865 870 875 880

Asn Ser Asp Ile Glu Ser Val Leu Phe Leu Ile Ser Val His Ala Asn
885 890 895

Val Asn Ser Arg Val Gln Asp Ala Ser Lys Leu Thr Pro Leu His Leu
900 905 910

Ala Val Gln Ala Gly Ser Glu Ile Ile Val Arg Asn Leu Leu Leu Ala
915 920 925

Gly Ala Lys Val Asn Glu Leu Thr Lys His Arg Gln Thr Ala Leu His
930 935 940

Leu Ala Ala Gln Gln Asp Leu Pro Thr Ile Cys Ser Val Leu Leu Glu
945 950 955 960

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Asn Gly Val Asp Phe Ala Ala Val Asp Glu Asn Gly Asn Asn Ala Leu
                965                 970                 975

His Leu Ala Val Met His Gly Arg Leu Asn Asn Ile Arg Val Leu Leu
            980                 985                 990

Thr Glu Cys Thr Val Asp Ala Glu Ala Phe Asn Leu Arg Gly Gln Ser
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Pro Leu His Ile Leu Gly Gln Tyr Gly Lys Glu Asn Ala Ala Ala Ile
   1010                1015                1020

Phe Asp Leu Phe Leu Glu Cys Met Pro Gly Tyr Pro Leu Asp Lys Pro
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Asp Ala Asp Gly Ser Thr Val Leu Leu Leu Ala Tyr Met Lys Gly Asn
                1045                1050                1055

Ala Asn Leu Cys Arg Ala Ile Val Arg Ser Gly Ala Arg Leu Gly Val
            1060                1065                1070

Asn Asn Asn Gln Gly Val Asn Ile Phe Asn Tyr Gln Val Ala Thr Lys
        1075                1080                1085

Gln Leu Leu Phe Arg Leu Leu Asp Met Leu Ser Lys Glu Pro Pro Trp
   1090                1095                1100

Cys Asp Gly Ser Tyr Cys Tyr Glu Cys Thr Ala Arg Phe Gly Val Thr
1105                1110                1115                1120

Thr Arg Lys His His Cys Arg His Cys Gly Arg Leu Leu Cys His Lys
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Cys Ser Thr Lys Glu Ile Pro Ile Ile Lys Phe Asp Leu Asn Lys Pro
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Val Arg Val Cys Asn Ile Cys Phe Asp Val Leu Thr Leu Gly Gly Val
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<212> DNA
<213> Homo sapiens

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<210> 4
<211> 1129
<212> PRT

<213> Homo sapiens

<400> 4

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            20                  25                  30

Arg Cys Ala Leu Leu Ala Ala Gln Ala Asn Lys Glu Ser Ser Ser Glu
        35                  40                  45

Ser Phe Ile Ser Arg Leu Leu Ala Ile Val Ala Asp Leu Tyr Glu Gln
    50                  55                  60

Glu Gln Tyr Ser Ala Asn Pro Glu Val Thr Met Thr Met Leu Arg Trp
65                  70                  75                  80
Ile Tyr Thr Asp Glu Leu Glu Phe Arg Glu Asp Asp Val Phe Leu Thr
                85                  90                  95

Glu Leu Met Lys Leu Ala Asn Arg Phe Gln Leu Gln Leu Leu Arg Glu
            100                 105                 110

Arg Cys Glu Lys Gly Val Met Ser Leu Val Asn Val Arg Asn Cys Ile
        115                 120                 125

Arg Phe Tyr Gln Thr Ala Glu Glu Leu Asn Ala Ser Thr Leu Met Asn
    130                 135                 140

Tyr Cys Ala Glu Ile Ile Ala Ser His Trp Asp Asp Leu Arg Lys Glu
145                 150                 155                 160

Asp Phe Ser Ser Met Ser Ala Gln Leu Leu Tyr Lys Met Ile Lys Ser
                165                 170                 175

Lys Thr Glu Tyr Pro Leu His Lys Ala Ile Lys Val Glu Arg Glu Asp
            180                 185                 190

Val Val Phe Leu Tyr Leu Ile Glu Met Asp Ser Gln Leu Pro Gly Lys
        195                 200                 205

Leu Asn Glu Ala Asp His Asn Gly Asp Leu Ala Leu Asp Leu Ala Leu
    210                 215                 220

Ser Arg Arg Leu Glu Ser Ile Ala Thr Thr Leu Val Ser His Lys Ala
225                 230                 235                 240

Asp Val Asp Met Val Asp Lys Ser Gly Trp Ser Leu Leu His Lys Gly
                245                 250                 255

Ile Gln Arg Gly Asp Leu Phe Ala Ala Thr Phe Leu Ile Lys Asn Gly
            260                 265                 270

Ala Phe Val Asn Ala Ala Thr Leu Gly Ala Gln Glu Thr Pro Leu His
        275                 280                 285
```

```
Leu Val Ala Leu Tyr Ser Ser Lys Lys His Ser Ala Asp Val Met Ser
    290                 295                 300

Glu Met Ala Gln Ile Ala Glu Ala Leu Leu Gln Ala Gly Ala Asn Pro
305                 310                 315                 320
Asn Met Gln Asp Ser Lys Gly Arg Thr Pro Leu His Val Ser Ile Met
                325                 330                 335

Ala Gly Asn Glu Tyr Val Phe Ser Gln Leu Leu Gln Cys Lys Gln Leu
            340                 345                 350

Asp Leu Glu Leu Lys Asp His Glu Gly Ser Thr Ala Leu Trp Leu Ala
        355                 360                 365

Val Gln His Ile Thr Val Ser Ser Asp Gln Ser Val Asn Pro Phe Glu
    370                 375                 380

Asp Val Pro Val Val Asn Gly Thr Ser Phe Asp Glu Asn Ser Phe Ala
385                 390                 395                 400

Ala Arg Leu Ile Gln Arg Gly Ser His Thr Asp Ala Pro Asp Thr Ala
                405                 410                 415

Thr Gly Asn Cys Leu Leu Gln Arg Ala Ala Gly Ala Gly Asn Glu Ala
            420                 425                 430

Ala Ala Leu Phe Leu Ala Thr Asn Gly Ala His Val Asn His Arg Asn
        435                 440                 445

Lys Trp Gly Glu Thr Pro Leu His Thr Ala Cys Arg His Gly Leu Ala
    450                 455                 460

Asn Leu Thr Ala Glu Leu Leu Gln Gln Gly Ala Asn Pro Asn Leu Gln
465                 470                 475                 480

Thr Glu Glu Ala Leu Pro Leu Pro Lys Glu Ala Ala Ser Leu Thr Ser
                485                 490                 495

Leu Ala Asp Ser Val His Leu Gln Thr Pro Leu His Met Ala Ile Ala
            500                 505                 510

Tyr Asn His Pro Asp Val Val Ser Val Ile Leu Glu Gln Lys Ala Asn
        515                 520                 525

Ala Leu His Ala Thr Asn Asn Leu Gln Ile Ile Pro Asp Phe Ser Leu
    530                 535                 540

Lys Asp Ser Arg Asp Gln Thr Val Leu Gly Leu Ala Leu Trp Thr Gly
545                 550                 555                 560
Met His Thr Ile Ala Ala Gln Leu Leu Gly Ser Gly Ala Ala Ile Asn
                565                 570                 575

Asp Thr Met Ser Asp Gly Gln Thr Leu Leu His Met Ala Ile Gln Arg
            580                 585                 590
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Gln Asp Ser Lys Ser Ala Leu Phe Leu Leu Glu His Gln Ala Asp Ile
        595                 600                 605

Asn Val Arg Thr Gln Asp Gly Glu Thr Ala Leu Gln Leu Ala Ile Arg
    610                 615                 620

Asn Gln Leu Pro Leu Val Val Asp Ala Ile Cys Thr Arg Gly Ala Asp
625                 630                 635                 640

Met Ser Val Pro Asp Glu Lys Gly Asn Pro Pro Leu Trp Leu Ala Leu
                645                 650                 655

Ala Asn Asn Leu Glu Asp Ile Ala Ser Thr Leu Val Arg His Gly Cys
            660                 665                 670

Asp Ala Thr Cys Trp Gly Pro Gly Pro Gly Gly Cys Leu Gln Thr Leu
        675                 680                 685

Leu His Arg Ala Ile Asp Glu Asn Asn Glu Pro Thr Ala Cys Phe Leu
    690                 695                 700

Ile Arg Ser Gly Cys Asp Val Asn Ser Pro Arg Gln Pro Gly Ala Asn
705                 710                 715                 720

Gly Glu Gly Glu Glu Glu Ala Arg Asp Gly Gln Thr Pro Leu His Leu
                725                 730                 735

Ala Ala Ser Trp Gly Leu Glu Glu Thr Val Gln Cys Leu Leu Glu Phe
            740                 745                 750

Gly Ala Asn Val Asn Ala Gln Asp Ala Glu Gly Arg Thr Pro Ile His
        755                 760                 765

Val Ala Ile Ser Ser Gln His Gly Val Ile Ile Gln Leu Leu Val Ser
    770                 775                 780

His Pro Asp Ile His Leu Asn Val Arg Asp Arg Gln Gly Leu Thr Pro
785                 790                 795                 800
Phe Ala Cys Ala Met Thr Phe Lys Asn Asn Lys Ser Ala Glu Ala Ile
                805                 810                 815

Leu Lys Arg Glu Ser Gly Ala Ala Glu Gln Val Asp Asn Lys Gly Arg
            820                 825                 830

Asn Phe Leu His Val Ala Val Gln Asn Ser Asp Ile Glu Ser Val Leu
        835                 840                 845

Phe Leu Ile Ser Val His Ala Asn Val Asn Ser Arg Val Gln Asp Ala
    850                 855                 860

Ser Lys Leu Thr Pro Leu His Leu Ala Val Gln Ala Gly Ser Glu Ile
865                 870                 875                 880

Ile Val Arg Asn Leu Leu Leu Ala Gly Ala Lys Val Asn Glu Leu Thr
```

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                885                 890                 895

Lys His Arg Gln Thr Ala Leu His Leu Ala Ala Gln Gln Asp Leu Pro
            900                 905                 910

Thr Ile Cys Ser Val Leu Leu Glu Asn Gly Val Asp Phe Ala Ala Val
        915                 920                 925

Asp Glu Asn Gly Asn Asn Ala Leu His Leu Ala Val Met His Gly Arg
    930                 935                 940

Leu Asn Asn Ile Arg Val Leu Leu Thr Glu Cys Thr Val Asp Ala Glu
945                 950                 955                 960

Ala Phe Asn Leu Arg Gly Gln Ser Pro Leu His Ile Leu Gly Gln Tyr
                965                 970                 975

Gly Lys Glu Asn Ala Ala Ala Ile Phe Asp Leu Phe Leu Glu Cys Met
            980                 985                 990

Pro Gly Tyr Pro Leu Asp Lys Pro Asp Ala Asp Gly Ser Thr Val Leu
        995                1000                1005

Leu Leu Ala Tyr Met Lys Gly Asn Ala Asn Leu Cys Arg Ala Ile Val
   1010                1015                1020

Arg Ser Gly Ala Arg Leu Gly Val Asn Asn Asn Gln Gly Val Asn Ile
1025                1030                1035                1040
Phe Asn Tyr Gln Val Ala Thr Lys Gln Leu Leu Phe Arg Leu Leu Asp
                1045                1050                1055

Met Leu Ser Lys Glu Pro Pro Trp Cys Asp Gly Ser Tyr Cys Tyr Glu
            1060                1065                1070

Cys Thr Ala Arg Phe Gly Val Thr Thr Arg Lys His His Cys Arg His
       1075                1080                1085

Cys Gly Arg Leu Leu Cys His Lys Cys Ser Thr Lys Glu Ile Pro Ile
   1090                1095                1100

Ile Lys Phe Asp Leu Asn Lys Pro Val Arg Val Cys Asn Ile Cys Phe
1105                1110                1115                1120

Asp Val Leu Thr Leu Gly Gly Val Ser
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<211> 2406
<212> DNA
<213> Homo sapiens

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<210> 6
<211> 784
<212> PRT
<213> Homo sapiens

<400> 6

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Tyr Glu Glu Glu His Ser Gly Glu Asp Arg Asp Val Lys Gly Gln Ile
         35                  40                  45
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```
Lys Ser Leu Val Gln Lys Ala Lys Lys Ala Lys Asp Arg Leu Leu Lys
 50                  55                  60

Arg Glu Gly Asp Asp Arg Ala Glu Ser Gly Thr Gln Gly Tyr Glu Ser
65                  70                  75                  80
Phe Ser Tyr Gly Gly Val Asp Pro Tyr Met Trp Glu Pro Gln Glu Leu
                85                  90                  95

Gly Ala Val Arg Ser His Leu Ser Asp Phe Lys Lys His Arg Ala Ala
            100                 105                 110

Arg Ile Asp His Tyr Val Val Glu Val Asn Lys Leu Ile Ile Arg Leu
        115                 120                 125

Glu Lys Leu Thr Ala Phe Asp Arg Thr Asn Thr Glu Ser Ala Lys Ile
    130                 135                 140

Arg Ala Ile Glu Lys Ser Val Val Pro Trp Val Asn Asp Gln Asp Val
145                 150                 155                 160

Pro Phe Cys Pro Asp Cys Gly Asn Lys Phe Ser Ile Arg Asn Arg Arg
                165                 170                 175

His His Cys Arg Leu Cys Gly Ser Ile Met Cys Lys Lys Cys Met Glu
            180                 185                 190

Leu Ile Ser Leu Pro Leu Ala Asn Lys Leu Thr Ser Ala Ser Lys Glu
        195                 200                 205

Ser Leu Ser Thr His Thr Ser Pro Ser Gln Ser Pro Asn Ser Val His
    210                 215                 220

Gly Ser Arg Arg Gly Ser Ile Ser Ser Met Ser Ser Val Ser Ser Val
225                 230                 235                 240

Leu Asp Glu Lys Asp Asp Asp Arg Ile Arg Cys Cys Thr His Cys Lys
                245                 250                 255

Asp Thr Leu Leu Lys Arg Glu Gln Gln Ile Asp Glu Lys Glu His Thr
            260                 265                 270

Pro Asp Ile Val Lys Leu Tyr Glu Lys Leu Arg Leu Cys Met Glu Lys
        275                 280                 285

Val Asp Gln Lys Ala Pro Glu Tyr Ile Arg Met Ala Ala Ser Leu Asn
    290                 295                 300

Ala Gly Glu Thr Thr Tyr Ser Leu Glu His Ala Ser Asp Leu Arg Val
305                 310                 315                 320
Glu Val Gln Lys Val Tyr Glu Leu Ile Asp Ala Leu Ser Lys Lys Ile
                325                 330                 335

Leu Thr Leu Gly Leu Asn Gln Asp Pro Pro Pro His Pro Ser Asn Leu
            340                 345                 350
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Arg Leu Gln Arg Met Ile Arg Tyr Ser Ala Thr Leu Phe Val Gln Glu
        355                 360                 365

Lys Leu Leu Gly Leu Met Ser Leu Pro Thr Lys Glu Gln Phe Glu Glu
    370                 375                 380

Leu Lys Lys Lys Arg Lys Glu Glu Met Glu Arg Lys Arg Xaa Val Glu
385                 390                 395                 400

Arg Gln Ala Ala Leu Glu Ser Gln Arg Arg Leu Glu Glu Arg Gln Ser
                405                 410                 415

Gly Leu Ala Ser Arg Ala Ala Asn Gly Glu Val Ala Ser Leu Arg Arg
            420                 425                 430

Gly Pro Ala Pro Leu Arg Lys Ala Glu Gly Trp Leu Pro Leu Ser Gly
        435                 440                 445

Gly Gln Gly Gln Ser Glu Asp Ser Asp Pro Leu Leu Gln Gln Ile His
    450                 455                 460

Asn Ile Thr Ser Phe Ile Arg Gln Ala Lys Ala Ala Gly Arg Met Asp
465                 470                 475                 480

Glu Val Arg Thr Leu Gln Glu Xaa Leu Arg Gln Leu Gln Asp Glu Tyr
                485                 490                 495

Asp Gln Gln Gln Thr Glu Lys Ala Ile Glu Leu Ser Arg Arg Gln Ala
            500                 505                 510

Glu Glu Glu Asp Leu Gln Arg Glu Gln Leu Gln Met Leu Arg Glu Arg
        515                 520                 525

Glu Leu Glu Arg Glu Arg Glu Gln Phe Arg Val Ala Ser Leu His Thr
    530                 535                 540

Arg Thr Arg Ser Leu Asp Phe Arg Glu Ile Gly Pro Phe Gln Leu Glu
545                 550                 555                 560
Pro Ser Arg Glu Pro Arg Thr His Leu Ala Tyr Ala Leu Asp Leu Gly
                565                 570                 575

Ser Ser Pro Val Pro Ser Ser Thr Ala Pro Lys Thr Pro Ser Leu Ser
            580                 585                 590

Ser Thr Gln Pro Thr Arg Val Trp Ser Gly Pro Pro Ala Val Gly Gln
        595                 600                 605

Glu Arg Leu Pro Gln Ser Ser Met Pro Gln Gln His Glu Gly Pro Ser
    610                 615                 620

Leu Asn Pro Phe Asp Glu Glu Asp Leu Ser Ser Pro Met Glu Glu Ala
625                 630                 635                 640

Thr Thr Gly Pro Pro Ala Ala Gly Val Ser Leu Asp Pro Ser Ala Arg
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Ile Leu Lys Glu Tyr Asn Pro Phe Glu Glu Glu Asp Glu Glu Glu Glu
            660                 665                 670

Ala Val Ala Gly Asn Pro Phe Ile Gln Pro Asp Ser Pro Ala Pro Asn
        675                 680                 685

Pro Phe Ser Glu Glu Asp Glu His Pro Gln Gln Arg Leu Ser Ser Pro
    690                 695                 700

Leu Val Pro Gly Asn Pro Phe Glu Glu Pro Thr Cys Ile Asn Pro Phe
705                 710                 715                 720

Glu Met Asp Ser Asp Ser Gly Pro Glu Ala Glu Glu Pro Ile Glu Glu
                725                 730                 735

Glu Leu Leu Leu Gln Gln Ile Asp Asn Ile Lys Ala Tyr Ile Phe Asp
            740                 745                 750

Ala Lys Gln Cys Gly Arg Leu Asp Glu Val Glu Val Leu Thr Glu Asn
        755                 760                 765

Leu Arg Glu Leu Lys His Thr Leu Ala Lys Gln Lys Gly Gly Thr Asp
    770                 775                 780
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<210> 7
<211> 1659
<212> DNA
<213> Homo sapiens

<400> 7

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ctcttaaatt tggaacataa ttactttgtt agtatctcag aactgattgg tgaaagagat 480
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cagcaagacc tagaaaataa atggtcgaat gaattaaaac aatcaactgc catccaaaaa 660
caagagtatc aagaatgggt aataaaactt caccaagacc taaaaaaccc caacaacagc 720
tcccttagtg aggaaattaa agttcagcca agtcagttca gagaatctgt agaagcaatt 780
ggaaggattt atgaggaaca gagaaagtta gaagaaagtt ttaccattca cttaggagcc 840
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aagaatgctg agaggaacta tttaaatgtt ttacctgggg aattttatat tacacggcat 1260
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tctaatctct cagaaatcca tgttgctttc catctctgtg tggatgacca tgtgaaatcg 1320
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gaaatgacta taccctggtg cttaaggaga gcggaacttg tgttcaagtg tgtcaaaggt 1500
ttcatgatgg aaatggcttc atgggatgga ggaatttcta ggacagtgca atttctagta 1560
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<210> 8
<211> 552
<212> PRT
<213> Homo sapiens

<400> 8

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Met Lys Lys Asn Arg Glu Arg Phe Cys Asn Arg Glu Arg Glu Phe Val
  1               5                  10                  15
Tyr Lys Phe Lys Val Gly Ser Gln Cys Leu Glu Leu Arg Val Pro Leu
             20                  25                  30

Lys Phe Pro Val Gln Glu Asn Ala Ser His Leu His Gly Arg Leu Met
         35                  40                  45

Leu Leu His Ser Leu Pro Cys Phe Ile Glu Lys Asp Leu Lys Glu Ala
     50                  55                  60

Leu Thr Gln Phe Ile Glu Glu Glu Ser Leu Ser Asp Tyr Asp Arg Asp
 65                  70                  75                  80

Ala Glu Ala Ser Leu Ala Ala Val Lys Ser Gly Glu Val Asp Leu His
                 85                  90                  95

Gln Leu Ala Ser Thr Trp Ala Lys Ala Tyr Ala Glu Thr Thr Leu Glu
            100                 105                 110

His Ala Arg Pro Glu Glu Pro Ser Trp Asp Glu Asp Phe Ala Asp Val
        115                 120                 125

Tyr His Asp Leu Ile His Ser Pro Ala Ser Glu Thr Leu Leu Asn Leu
    130                 135                 140

Glu His Asn Tyr Phe Val Ser Ile Ser Glu Leu Ile Gly Glu Arg Asp
145                 150                 155                 160

Val Glu Leu Lys Lys Leu Arg Glu Arg Gln Gly Ile Glu Met Glu Lys
                165                 170                 175

Val Met Gln Glu Leu Gly Lys Ser Leu Thr Asp Gln Asp Val Asn Ser
            180                 185                 190

Leu Ala Ala Gln His Phe Glu Ser Gln Gln Asp Leu Glu Asn Lys Trp
        195                 200                 205

Ser Asn Glu Leu Lys Gln Ser Thr Ala Ile Gln Lys Gln Glu Tyr Gln
    210                 215                 220
```

Glu Trp Val Ile Lys Leu His Gln Asp Leu Lys Asn Pro Asn Asn Ser
225 230 235 240

Ser Leu Ser Glu Glu Ile Lys Val Gln Pro Ser Gln Phe Arg Glu Ser
245 250 255

Val Glu Ala Ile Gly Arg Ile Tyr Glu Glu Gln Arg Lys Leu Glu Glu
260 265 270

Ser Phe Thr Ile His Leu Gly Ala Gln Leu Lys Thr Met His Asn Leu
275 280 285

Arg Leu Leu Arg Ala Asp Met Leu Asp Phe Cys Lys His Lys Arg Asn
290 295 300

His Arg Ser Gly Val Lys Leu His Arg Leu Gln Thr Ala Leu Ser Leu
305 310 315 320

Tyr Ser Thr Ser Leu Cys Gly Leu Val Leu Leu Val Asp Asn Arg Ile
325 330 335

Asn Ser Tyr Ser Gly Ile Lys Arg Asp Phe Ala Thr Val Cys Gln Glu
340 345 350

Cys Thr Asp Phe His Phe Pro Arg Ile Glu Glu Gln Leu Glu Val Val
355 360 365

Gln Gln Val Val Leu Tyr Ala Arg Thr Gln Arg Arg Ser Lys Leu Lys
370 375 380

Glu Ser Leu Asp Ser Gly Asn Gln Asn Gly Gly Asn Asp Asp Lys Thr
385 390 395 400

Lys Asn Ala Glu Arg Asn Tyr Leu Asn Val Leu Pro Gly Glu Phe Tyr
405 410 415

Ile Thr Arg His Ser Asn Leu Ser Glu Ile His Val Ala Phe His Leu
420 425 430

Cys Val Asp Asp His Val Lys Ser Gly Asn Ile Thr Ala Arg Asp Pro
435 440 445

Ala Ile Met Gly Leu Arg Asn Ile Leu Lys Val Cys Cys Thr His Asp
450 455 460

Ile Thr Thr Ile Ser Ile Pro Leu Leu Leu Val His Asp Met Ser Glu
465 470 475 480

Glu Met Thr Ile Pro Trp Cys Leu Arg Arg Ala Glu Leu Val Phe Lys
485 490 495

Cys Val Lys Gly Phe Met Met Glu Met Ala Ser Trp Asp Gly Gly Ile
500 505 510

Ser Arg Thr Val Gln Phe Leu Val Pro Gln Ser Ile Ser Glu Glu Met
515 520 525

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Phe Tyr Gln Leu Ser Asn Met Leu Pro Gln Ile Phe Arg Val Ser Ser
    530                 535                 540

Thr Leu Thr Leu Thr Ser Lys His
545                 550
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<210> 9
<211> 1050
<212> DNA
<213> Homo sapiens

<400> 9
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gctctgagcc agataaatac aaagcttctg gcagaaatga agatgaaaaa ggatttattt 180
cctgttggga gagaaattgc tggaattgta ttagatgttg gaagcaaggt atcattcttt 240
caaccagatg atgaagtagt tggaattttg cccctggact ctgaagaccc tggactttgt 300
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<210> 10
<211> 349
<212> PRT
<213> Homo sapiens

<400> 10
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Met Lys Gly Leu Tyr Phe Gln Gln Ser Ser Thr Asp Glu Glu Ile Thr
  1               5                  10                  15

Phe Val Phe Gln Glu Lys Glu Asp Leu Pro Val Thr Glu Asp Asn Phe
             20                  25                  30

Val Lys Leu Gln Val Lys Ala Cys Ala Leu Ser Gln Ile Asn Thr Lys
         35                  40                  45

Leu Leu Ala Glu Met Lys Met Lys Lys Asp Leu Phe Pro Val Gly Arg
     50                  55                  60

Glu Ile Ala Gly Ile Val Leu Asp Val Gly Ser Lys Val Ser Phe Phe
 65                  70                  75                  80
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Gln Pro Asp Asp Glu Val Val Gly Ile Leu Pro Leu Asp Ser Glu Asp
85 90 95

Pro Gly Leu Cys Glu Val Val Arg Val His Glu His Tyr Leu Val His
100 105 110

Lys Pro Glu Lys Val Thr Trp Thr Glu Ala Ala Gly Ser Ile Arg Asp
115 120 125

Gly Val Arg Ala Tyr Thr Ala Leu His Tyr Leu Ser His Leu Ser Pro
130 135 140

Gly Lys Ser Val Leu Ile Met Asp Gly Ala Ser Ala Phe Gly Thr Ile
145 150 155 160

Ala Ile Gln Leu Ala His His Arg Gly Ala Lys Val Ile Ser Thr Ala
165 170 175

Cys Ser Leu Glu Asp Lys Gln Cys Leu Glu Arg Phe Arg Pro Pro Ile
180 185 190

Ala Arg Val Ile Asp Val Ser Asn Gly Lys Val His Val Ala Glu Ser
195 200 205

Cys Leu Glu Glu Thr Gly Gly Leu Gly Val Asp Ile Val Leu Asp Ala
210 215 220

Gly Val Arg Leu Tyr Ser Lys Asp Asp Glu Pro Ala Val Lys Leu Gln
225 230 235 240

Leu Leu Pro His Lys His Asp Ile Ile Thr Leu Leu Gly Val Gly Gly
245 250 255

His Trp Val Thr Thr Glu Glu Asn Leu Gln Leu Asp Pro Pro Asp Ser
260 265 270

His Cys Leu Phe Leu Lys Gly Ala Thr Leu Ala Phe Leu Asn Asp Glu
275 280 285

Val Trp Asn Leu Ser Asn Val Gln Gln Gly Lys Tyr Leu Cys Ile Leu
290 295 300

Lys Asp Val Met Glu Lys Leu Ser Thr Gly Val Phe Arg Pro Gln Leu
305 310 315 320

Asp Glu Pro Ile Pro Leu Tyr Glu Ala Lys Val Ser Met Glu Ala Val
325 330 335

Gln Lys Asn Gln Gly Arg Lys Lys Gln Val Val Gln Phe
340 345

<210> 11
<211> 663

<212> DNA
<213> Homo sapiens

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ctgcaggtgg ccacccctgg ggggaaagcc atggaatttg tggatgtgac tgagagcaat 180
gcacgctggg tgcaagactt ccgcctcaag gcttacgcca gccccgccaa gctcgagtcc 240
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tga 663

<210> 12
<211> 220
<212> PRT
<213> Homo sapiens

<400> 12
Met Ala Ser Glu Arg Leu Pro Asn Arg Pro Ala Cys Leu Leu Val Ala
1 5 10 15

Ser Gly Ala Ala Glu Gly Val Ser Ala Gln Ser Phe Leu His Cys Phe
20 25 30

Thr Met Ala Ser Thr Ala Phe Asn Leu Gln Val Ala Thr Pro Gly Gly
35 40 45

Lys Ala Met Glu Phe Val Asp Val Thr Glu Ser Asn Ala Arg Trp Val
50 55 60

Gln Asp Phe Arg Leu Lys Ala Tyr Ala Ser Pro Ala Lys Leu Glu Ser
65 70 75 80

Ile Asp Gly Ala Arg Tyr His Ala Leu Leu Ile Pro Ser Cys Pro Gly
85 90 95

Ala Leu Thr Asp Leu Ala Ser Ser Gly Ser Leu Ala Arg Ile Leu Gln
100 105 110

His Phe His Ser Glu Ser Lys Pro Ile Cys Ala Val Gly His Gly Val
115 120 125

Ala Ala Leu Cys Cys Ala Thr Asn Glu Asp Arg Ser Trp Val Phe Asp
130 135 140

Ser Tyr Ser Leu Thr Gly Pro Ser Val Cys Glu Leu Val Arg Ala Pro
145 150 155 160

Gly Phe Ala Arg Leu Pro Leu Val Val Glu Asp Phe Val Lys Asp Ser
165 170 175

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Gly Ala Cys Phe Ser Gly Leu Gly Ala Ala Pro Gly Trp Gly Arg Gly
            180                 185                 190

Gly Lys Gln Glu Asp Arg Pro Pro Glu Glu Gln Arg Arg Ala Gly Glu
        195                 200                 205
Asp Phe Pro Ile Tyr Gln Cys Val Phe Ile Tyr Pro
    210                 215                 220


<210> 13
<211> 2679
<212> DNA
<213> Homo sapiens

<400> 13
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cgctttcaaa tccttaaaac catcacccat cccagactct gccagtatgt ggatatttct 180
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<210> 14
<211> 892
<212> PRT
<213> Homo sapiens

<400> 14
Met Phe Pro Leu Lys Asp Ala Glu Met Gly Ala Phe Thr Phe Phe Ala
1 5 10 15

Ser Ala Leu Pro His Asp Val Cys Gly Ser Asn Gly Leu Pro Leu Thr
20 25 30

Pro Asn Ser Ile Lys Ile Leu Gly Arg Phe Gln Ile Leu Lys Thr Ile
35 40 45

Thr His Pro Arg Leu Cys Gln Tyr Val Asp Ile Ser Arg Gly Lys His
50 55 60

Glu Arg Leu Val Val Val Ala Glu His Cys Glu Arg Ser Leu Glu Asp
65 70 75 80

Leu Leu Arg Glu Arg Lys Pro Val Ser Cys Ser Thr Val Leu Cys Ile
85 90 95

Ala Phe Glu Val Leu Gln Gly Leu Gln Tyr Met Asn Lys His Gly Ile
100 105 110

Val His Arg Ala Leu Ser Pro His Asn Ile Leu Leu Asp Arg Lys Gly
115 120 125

His Ile Lys Leu Ala Lys Phe Gly Leu Tyr His Met Thr Ala His Gly
130 135 140

Asp Asp Val Asp Phe Pro Ile Gly Tyr Pro Ser Tyr Leu Ala Pro Glu
145 150 155 160

Val Ile Ala Gln Gly Ile Phe Lys Thr Thr Asp His Met Pro Ser Lys
165 170 175

Lys Pro Leu Pro Ser Gly Pro Lys Ser Asp Val Trp Ser Leu Gly Ile
180 185 190

Ile Leu Phe Glu Leu Cys Val Gly Arg Lys Leu Phe Gln Ser Leu Asp
195 200 205

Ile Ser Glu Arg Leu Lys Phe Leu Leu Thr Leu Asp Cys Val Asp Asp

```
     210                 215                 220

Thr Leu Ile Val Leu Ala Glu Glu His Gly Cys Leu Asp Ile Ile Lys
225                 230                 235                 240

Glu Leu Pro Glu Thr Val Ile Asp Leu Leu Asn Lys Cys Leu Thr Phe
                245                 250                 255

His Pro Ser Lys Arg Pro Thr Pro Asp Glu Leu Met Lys Asp Lys Val
            260                 265                 270

Phe Ser Glu Val Ser Pro Leu Tyr Thr Pro Phe Thr Lys Pro Ala Ser
        275                 280                 285

Leu Phe Ser Ser Ser Leu Arg Cys Ala Asp Leu Thr Leu Pro Glu Asp
    290                 295                 300

Ile Ser Gln Leu Cys Lys Asp Ile Asn Asn Asp Tyr Leu Ala Glu Arg
305                 310                 315                 320

Ser Ile Glu Glu Val Tyr Tyr Leu Trp Cys Leu Ala Gly Gly Asp Leu
                325                 330                 335

Glu Lys Glu Leu Val Asn Lys Glu Ile Ile Arg Ser Lys Pro Pro Ile
            340                 345                 350

Cys Thr Leu Pro Asn Phe Leu Phe Glu Asp Gly Glu Ser Phe Gly Gln
        355                 360                 365
Gly Arg Asp Arg Ser Ser Leu Leu Asp Asp Thr Thr Val Thr Leu Ser
    370                 375                 380

Leu Cys Gln Leu Arg Asn Arg Leu Lys Asp Val Gly Gly Glu Ala Phe
385                 390                 395                 400

Tyr Pro Leu Leu Glu Asp Asp Gln Ser Asn Leu Pro His Ser Asn Ser
                405                 410                 415

Asn Asn Glu Leu Ser Ala Ala Ala Met Leu Pro Leu Ile Ile Arg Glu
            420                 425                 430

Lys Asp Thr Glu Tyr Gln Leu Asn Arg Ile Ile Leu Phe Asp Arg Leu
        435                 440                 445

Lys Ala Tyr Pro Tyr Lys Lys Asn Gln Ile Trp Lys Glu Ala Arg Val
    450                 455                 460

Asp Ile Pro Pro Leu Met Arg Gly Leu Thr Trp Ala Ala Leu Leu Gly
465                 470                 475                 480

Val Glu Gly Ala Ile His Ala Lys Tyr Asp Ala Ile Asp Lys Asp Thr
                485                 490                 495

Pro Ile Pro Thr Asp Arg Gln Ile Glu Val Asp Ile Pro Arg Cys His
            500                 505                 510
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[redacted]

Gln Tyr Asp Glu Leu Leu Ser Ser Pro Glu Gly His Ala Lys Phe Arg
515 520 525

Arg Val Leu Lys Ala Trp Val Val Ser His Pro Asp Leu Val Tyr Trp
530 535 540

Gln Gly Leu Asp Ser Leu Cys Ala Pro Phe Leu Tyr Leu Asn Phe Asn
545 550 555 560

Asn Glu Ala Leu Ala Tyr Ala Cys Met Ser Ala Phe Ile Pro Lys Tyr
565 570 575

Leu Tyr Asn Phe Phe Leu Lys Asp Asn Ser His Val Ile Gln Glu Tyr
580 585 590

Leu Thr Val Phe Ser Gln Met Ile Ala Phe His Asp Pro Glu Leu Ser
595 600 605
Asn His Leu Asn Gln Ile Gly Phe Ile Pro Asp Leu Tyr Ala Ile Pro
610 615 620

Trp Phe Leu Thr Met Phe Thr His Val Phe Pro Leu His Lys Ile Phe
625 630 635 640

His Leu Trp Asp Thr Leu Leu Leu Gly Asn Ser Ser Phe Pro Phe Cys
645 650 655

Ile Gly Val Ala Ile Leu Gln Gln Leu Arg Asp Arg Leu Leu Ala Asn
660 665 670

Gly Phe Asn Glu Cys Ile Leu Leu Phe Ser Asp Leu Pro Glu Ile Asp
675 680 685

Ile Glu Arg Cys Val Arg Glu Ser Ile Asn Leu Phe Cys Trp Thr Pro
690 695 700

Lys Ser Ala Thr Tyr Arg Gln His Ala Gln Pro Pro Lys Pro Ser Ser
705 710 715 720

Asp Ser Ser Gly Gly Arg Ser Ser Ala Pro Tyr Phe Ser Ala Glu Cys
725 730 735

Pro Asp Pro Pro Lys Thr Asp Leu Ser Arg Glu Ser Ile Pro Leu Asn
740 745 750

Asp Leu Lys Ser Glu Val Ser Pro Arg Ile Ser Ala Glu Asp Leu Ile
755 760 765

Asp Leu Cys Glu Leu Thr Val Thr Gly His Phe Lys Thr Pro Ser Lys
770 775 780

Lys Thr Lys Ser Ser Lys Pro Lys Leu Leu Val Val Asp Ile Leu Asn
785 790 795 800

Ser Glu Asp Phe Ile Arg Gly His Ile Ser Gly Ser Ile Asn Ile Pro
805 810 815

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Phe Ser Ala Ala Phe Thr Ala Glu Gly Glu Leu Thr Gln Gly Pro Tyr
            820                 825                 830

Thr Ala Met Leu Gln Asn Phe Lys Gly Lys Val Ile Val Ile Val Gly
        835                 840                 845
His Val Ala Lys His Thr Ala Glu Phe Ala Ala His Leu Val Lys Met
    850                 855                 860

Lys Tyr Pro Arg Ile Cys Ile Leu Asp Gly Gly Ile Asn Lys Ile Lys
865                 870                 875                 880

Pro Thr Gly Leu Leu Thr Ile Pro Ser Pro Gln Ile
                885                 890
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<210> 15
<211> 1752
<212> DNA
<213> Homo sapiens

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gctctcaatg ttcccctgca caataggcga caccagctga agatgcgaga tatagctggg 180
caggccctgg cttttgttca ggatcttgta ccggctcttc tgaactttca cacctataca 240
gaacagagga ttcaaatttt ccctgttgat tctgccatag acactatatc accattgaat 300
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aaattgaaaa tgttttcaga tcatctaact tcctacgtac gcttcctgag gaagatcctt 480
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atgaaagata tttccaaaca ctatagtcag aaggcttcca tagagcatga aattccaaca 780
gcaacacaga agctagtaac gactaacgac tgcatcttgt cctcagcggt gacgttaaca 840
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             20                  25                  30

Asp Thr Lys Cys His Leu Tyr Asn Ala Leu Asn Val Pro Leu His Asn
         35                  40                  45

Arg Arg His Gln Leu Lys Met Arg Asp Ile Ala Gly Gln Ala Leu Ala
     50                  55                  60

Phe Val Gln Asp Leu Val Pro Ala Leu Leu Asn Phe His Thr Tyr Thr
 65                  70                  75                  80

Glu Gln Arg Ile Gln Ile Phe Pro Val Asp Ser Ala Ile Asp Thr Ile
                 85                  90                  95

Ser Pro Leu Asn Gln Lys Phe Ser Gln Tyr Leu His Glu Asn Ala Ser
            100                 105                 110

Tyr Val Arg Pro Leu Glu Glu Gly Met Leu His Leu Phe Glu Ser Ile
        115                 120                 125

Thr Glu Asp Thr Val Thr Val Leu Glu Thr Thr Val Lys Leu Lys Met
    130                 135                 140

Phe Ser Asp His Leu Thr Ser Tyr Val Arg Phe Leu Arg Lys Ile Leu
145                 150                 155                 160

Pro Tyr Gln Leu Lys Ser Leu Glu Glu Glu Cys Glu Ser Ser Leu Cys
                165                 170                 175

Thr Pro Ala Leu Arg Ala Arg Asn Leu Glu Leu Ser Gln Asp Met Lys
            180                 185                 190

Thr Met Thr Ala Val Phe Glu Lys Leu Gln Thr Tyr Val Thr Leu Leu
        195                 200                 205

Ala Leu Pro Ser Thr Glu Pro Asp Gly Leu Leu Arg Thr Asn Tyr Thr
    210                 215                 220

Ser Val Leu Thr Asn Val Gly Ala Ala Leu His Gly Phe His Asp Val
225                 230                 235                 240

Met Lys Asp Ile Ser Lys His Tyr Ser Gln Lys Ala Ser Ile Glu His
                245                 250                 255

Glu Ile Pro Thr Ala Thr Gln Lys Leu Val Thr Thr Asn Asp Cys Ile
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260 265 270

Leu Ser Ser Ala Val Thr Leu Thr Asn Gly Ala Gly Lys Ile Ala Ser
275 280 285

Phe Phe Gly Asn Asn Val Asp Tyr Phe Ile Ala Ser Leu Ser Tyr Gly
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Pro Lys Thr Ala Ser Gly Phe Ile Ser Pro Leu Ser Ala Glu Cys Met
305 310 315 320

Leu Gln Tyr Lys Lys Lys Ala Ala Ala Tyr Met Lys Ser Leu Arg Thr
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Pro Leu Ala Glu Ser Val Pro Tyr Gly Glu Ala Val Ala Asn Arg Arg
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Val Leu Leu Ser Ser Thr Glu Ser Arg Glu Gly Leu Ala Gln Gln Val
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Gln Gln Ser Leu Glu Lys Ile Ser Lys Leu Glu Gln Glu Lys Glu His
370 375 380

Trp Met Leu Glu Ala Gln Leu Ala Lys Ile Lys Leu Glu Lys Glu Asn
385 390 395 400

Gln Arg Ile Ala Asp Arg Leu Arg Gly Thr Thr Ser Ala Gln Leu Pro
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Gly Leu Ala Gln Glu Asn Ala Thr Val Pro Ile Ala Ser Ser Gln Glu
420 425 430

Glu Ala Ala Ala Lys Val Leu Thr Glu Pro Val Gln Ser Thr Ser Leu
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Val Gly Met Leu Thr Arg Thr Pro Asp Ser Glu Ala Pro Asp Val Glu
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Ser Arg Glu Asp Leu Ile Lys Ser His Tyr Met Ala Arg Ile Ala Glu
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Leu Thr Ser Gln Leu Gln Leu Ala Asp Ser Lys Ser Val His Phe Tyr
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Ala Glu Cys Arg Ala Leu Ser Lys Arg Leu Ala Leu Ala Glu Lys Ser
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Lys Glu Thr Leu Thr Glu Glu Met Arg Leu Ala Ser Gln Asn Ile Ser
515 520 525

Arg Leu Gln Asp Glu Leu Met Thr Thr Lys Arg Ser Tyr Glu Asp Gln
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Leu Ser Met Met Ser Asp His Leu Cys Ser Met Asn Glu Thr Leu Ser
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Lys Gln Arg Glu Glu Ile Asp Thr Leu Lys Met Ala Ser Lys Gly Asn
565 570 575

Ser Lys Lys Thr Arg Asn Arg
580